RESPONSE OF DOGS TO LIVER EXTRACTS CONTAINING THE PERNICIOUS ANEMIA FACTOR[1]

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FOUR FIGURES

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Niacin was first shown to be essential for the dog through the use of the modified Goldberger diet (Elvehjem et al., '38). Schaefer et al. ('42) found that young puppies developed symptoms of blacktongue in 14–18 days when niacin was omitted from a purified ration containing sucrose, casein, cottonseed oil and cod liver oil supplemented with thiamine, riboflavin, pyridoxine, pantothenic acid and choline. The syndrome could be counteracted by the administration of niacin, but incomplete responses were observed in certain cases.

West ('41) first observed that sulfapyridine inhibited the curative effect of niacin in deficient dogs and that fresh liver counteracted this inhibition. Schaefer et al. ('42) repeated this work using the purified ration described above and found that the sulfapyridine inhibition could not be overcome by nicotinamide, dried liver or liver extract powder.

Krehl et al. ('45, '46) found that a more consistent response to niacin could be obtained when folic acid was added to the purified rations. Furthermore, these workers found that fresh

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liver and milk were effective in counteracting the sulfapyridine-induced inhibition in niacin-deficient animals.

Rhoades and Miller ('33) studied the anemia associated with blacktongue and found that a progressive macrocytic anemia developed in dogs which were failing on deficient diets. Handler and Featherston ('43) demonstrated the same type of anemia in dogs by feeding a modified Goldberger diet, the corn meal diet of Koehn and Elvehjem ('37) and a purified diet. The anemia in the case of dogs on the 1st two diets responded well to the administration of niacin or nicotinamide. However, the animals receiving the purified diet showed only suboptimal responses to niacin (10–12 gm % hemoglobin). Consequently, these workers suggested that other factors might be involved.

Krehl et al. ('45, '46) found that even though folic acid helped to produce more consistent responses to niacin on a niacin-deficient ration, the anemia which prevailed was little affected by the presence of folic acid. Because complete responses were not obtained with folic acid, these workers also suggest the possibility that another factor is involved.

In this report, we wish to present further evidence that an additional factor is required by the dog when maintained on a niacin-deficient diet. We also wish to present data to show that commercial pernicious anemia preparations are very rich sources of the factor.

METHODS

Approximately 35 weanling mongrel dogs were used in these experiments. They were dewormed and freed of external parasites at the beginning of the experiment, and were placed in individual cages equipped with heavy wire mesh bottoms. All animals were given the basal ration and water ad libitum. The basal ration consisted of sucrose 65%, alcohol-extracted casein 19%, cottonseed oil 11%, salts IV 4% and sulfasuxidine 1%. Some modifications in the composition of the basal ration were made, but these are indicated in each case. The following crystalline B vitamins were fed as sup-

plements to the basal ration: 0.1 mg each of thiamine and riboflavin, and 0.6, 0.5 and 25 mg, respectively, of pyridoxine hydrochloride, calcium pantothenate and choline chloride, per kg of body weight per day. The vitamins were prepared as an aqueous suspension at such a concentration that the suspension could be fed at the rate of 1 ml per kg of body weight per day on every 3rd day. In addition, 1800 I.U. of vitamin A and 500 I.U. of vitamin D per day were given.[2]

Blood samples (3 ml) were taken routinely from the radial vein, and hemoglobin values, red cell counts and hematocrit values were determined. From these data, the mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were calculated.

EXPERIMENTAL AND RESULTS

Young growing dogs lost weight when placed on a niacin-deficient ration, and niacin therapy became necessary in 14–18 days. In most cases, the animals gained between 800 and 1000 gm when given a single dose of 25 mg of niacin. This weight response was only temporary, however, and the animals soon began to lose weight again. The administration of a 2nd dose of 25 mg of niacin brought about weight responses in a number of dogs, but eventually a point was reached where all animals failed to respond to niacin (fig. 1). Unless further therapy was given, death resulted in these animals. The number of responses to single doses of niacin varied between 2 and 5 per dog. When the animals failed to respond to niacin, blood data revealed that an anemia persisted (8–12 gm % hemoglobin, 2–3 million red cells and hematocrit values of 20–30%). These data, which were collected from 20 animals, were used to calculate the mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration. Similar blood data from 20 control animals receiving niacin from the beginning of the experiment were taken simultaneously and the same calculations were made. These are summarized in table 1.

[2] Vitamin A and vitamin D given in the form of Haliver oil and Drisdol.

From these data it is clear that a macrocytic anemia existed in the animals which failed to respond to niacin.

Since folic acid had been shown to play a part in the response of dogs to niacin, it was fed at a level of 0.1 mg per day to 6 animals which had failed to respond to niacin. Some of the animals showed weight responses as a result of the folic

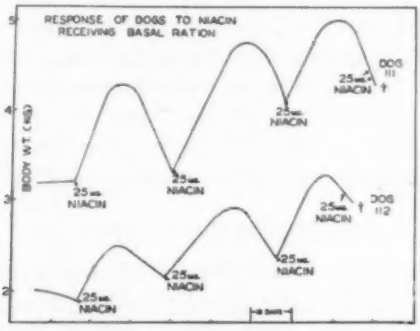

Fig. 1 Failure of dogs to respond to niacin.

TABLE 1

Comparison of blood pictures of control animals and depleted animals failing to respond to niacin

	MEAN CORP. VOLUME	MEAN CORP. HEMOGLOBIN	MEAN CORP. HEMOGLOBIN CONCENTRATION
	mm^3	$\mu\mu g$	%
Control animals	69	22.4	32.1
Depleted animals	82	27.5	33.8

acid therapy, but little if any change could be observed in the blood picture (table 2). Several animals developed a flaccid type of paralysis and died. An animal afflicted with typical paralysis is shown in figure 2. Whether or not this paralysis is related to that seen in humans suffering from pernicious anemia has not been determined. In general, the longer the animal can be maintained in the deficient state, the more severe the symptoms become. It is exceedingly difficult to

prolong the deficiency, however, since most of the dogs in this condition die very suddenly.

Since the paralysis and the anemia were similar to the symptoms encountered in pernicious anemia, and since stubborn cases of pellagra in the human are sometimes treated successfully with liver extracts, commercial preparations of the pernicious anemia factor were tried. Several dogs which

TABLE 2

Effects of folic acid and antipernicious anemia concentrates (A.P.A.) upon dogs failing to respond to niacin

DOG NO.	FOLIC ACID THERAPY					A. P. A. THERAPY		
	Body weight		Duration of test	Hemoglobin		Weight after therapy	Duration of test	Hemoglobin after therapy
	Before therapy	After therapy		Before therapy	After therapy			
	kg	*kg*	*days*	*gm%*	*gm%*	*kg*	*days*	*gm%*
114 [1]	9.2	9.0	30	10.89	11.1	10.5	60	15.15
120 [1]	9.1	6.5	18	11.71	11.28	+	..	.....
121 [1]	6.9	10.0	30	10.89	10.35	—	..	.....
419 [1]	8.6	8.4	5	11.55	11.55	+	..	.....
445 [1]	7.2	9.5	30	9.13	9.73	11.6	30	13.7
451 [1]	6.3	7.3	35	12.21	12.76	8.6	30	15.29
	A. P. A. THERAPY					FOLIC ACID THERAPY		
119 [2]	7.25	10.0	37	10.28	11.21	10.5	30	14.6
118 [3]	6.8	7.8	60	12.9	13.0	8.9	28	15.8
204 [2]	8.7	16.2	60	10.45	12.0	20.2	59	15.0

[1] Dogs 114, 120, 121, 419, 445, and 451 each received 1 U.S.P. unit of reticulogen (Lilly) injected per day.

[2] Dogs 119 and 204 received 10 and 7 U.S.P. units respectively of reticulogen per day.

[3] Dog 118 received 7 U.S.P. units of Sharp and Dohme no. 2505 per day, and was given B_c conjugate instead of free folic acid.

had failed to respond to niacin on a niacin-deficient ration were given adequate amounts of niacin and folic acid. Little weight and no blood responses were seen. These animals were required to show a weight and hematological plateau for a considerable length of time (approximately 1 month) before receiving injections of the liver extracts. One U.S.P. unit per day of either reticulogen [3] or another preparation [4] was

[3] Lilly.

[4] Sharp and Dohme no. 2505.

injected intramuscularly. Typical growth and hemoglobin curves for 2 animals receiving folic acid and liver extract are given in figure 3.

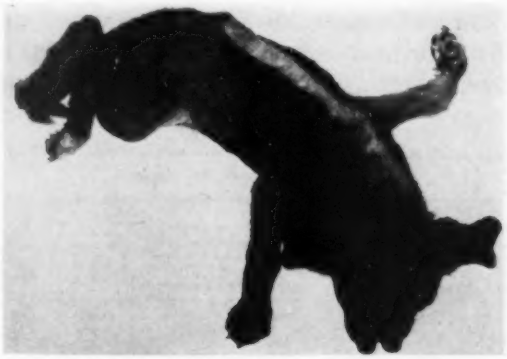

Fig. 2 A typical case of paralysis observed on a niacin-deficient diet.

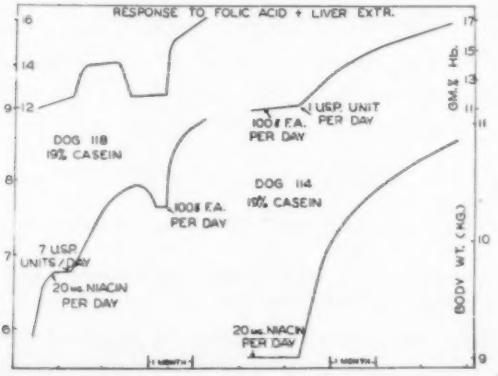

Fig. 3 Response of dogs to folic acid and liver extract.

In order to determine if folic acid was needed in this regimen, animals which had failed to respond to niacin and which were receiving adequate amounts of niacin were given comparable injections of the same liver preparations without folic acid. Some response in weight and hemoglobin occurred

(fig. 3) but these values soon returned to suboptimal levels. When folic acid was added, a complete response was seen, indicating that this factor is necessary.

When a basal ration containing 19% casein was fed, a need for folic acid could be shown. When higher levels of casein were fed, however, good responses to the pernicious anemia concentrates could be obtained without the addition of folic acid (fig. 4).

Folic acid conjugate was tried in one dog receiving the liver extract, and a response comparable to that seen in dogs receiving free folic acid was observed (fig. 3). Apparently

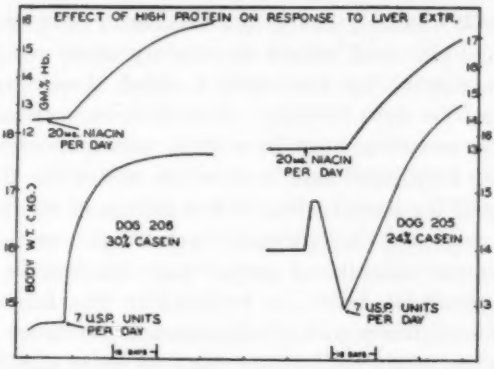
EFFECT OF HIGH PROTEIN ON RESPONSE TO LIVER EXTR.
GM.% Hb.
BODY WT. (KG.)
20mg. NIACIN PER DAY
20mg. NIACIN PER DAY
DOG 208 30% CASEIN
DOG 205 24% CASEIN
7 U.S.P. UNITS PER DAY
7 U.S.P. UNITS PER DAY

Fig. 4 Response to liver extracts of dogs receiving 24 and 30% protein rations.

the dog can utilize the bound form of folic acid, as contrasted to the monkey which, under our experimental conditions, cannot utilize the conjugated forms very effectively (Cooperman, Elvehjem, McCall and Ruegamer, '46).

Several attempts were made to develop an assay for the factor found in the liver extract. It was thought that by giving a single injection of the liver extract, a correlation between the weight or hemoglobin response and the U.S.P. unitage might be attained. It was found, however, that the animal apparently could not store the factor, since equal weight

responses were seen with the injection of several levels of the extract. Apparently only a given amount of the extract from a single injection can be utilized. In view of these results, daily injections were tried and it was found that optimal growth and hemoglobin production could be obtained with one U.S.P. unit of reticulogen injected per day. Lower levels were not tried. These results are summarized in table 2.

DISCUSSION

When a niacin-free basal ration containing 1% sulfasuxidine was fed to young growing dogs, the animals began to lose weight and developed signs of blacktongue in 14–18 days. Niacin was only partially effective in counteracting the syndrome. Folic acid helped to produce more consistent responses to niacin, but the anemia which developed did not respond to folic acid therapy. It should be pointed out that blood responses were seen in several animals receiving folic acid, but we attributed this to a failure to deplete the animals completely of the liver factor. When folic acid was withdrawn from the regimen, the animals lost weight and developed a more severe anemia. A point was reached in all cases where the animals failed to respond to the folic acid, but responded completely to the administration of liver extract.

There were many indications that we were dealing with a condition similar to pernicious anemia in the human. The anemia observed in the dog was found to be macrocytic-normochromic in nature, and it developed progressively. The animals often suffered from diarrhea and showed general lassitude. A flaccid type of paralysis was observed in several of the more advanced cases, and liver damage such as that described by Krehl et al. ('46) was also seen in some animals at autopsy.

As little as one U.S.P. unit of pernicious anemia activity was effective in bringing about a complete remission of the anemia observed in dogs which had failed to respond to niacin. In the case of reticulogen this would mean that $\frac{1}{20}$ ml

of the extract was injected per day, or that approximately 10 mg of solid material was sufficient to produce an optimal response in the dog. If we are not dealing with the pernicious anemia factor itself, then it must be something else that was concentrated to a high degree along with it.

The liver extracts were used first with adequate amounts of folic acid; when folic acid was omitted poor results were obtained with the extract. When folic acid was added, a complete response was seen, indicating that it was necessary. It would appear that all three factors — niacin, folic acid and the liver extract — are required. If any one of the three is present in inadequate amount, it is a limiting factor, and optimal growth and blood formation will not take place. This was true only when the protein level was about 19% because when the level was increased to 24 or 30% the need for folic acid could not be demonstrated. Possibly higher levels of casein favor the intestinal synthesis of folic acid in the dog.

Since the time this work was first instituted (Ruegamer et al., '47), liver extracts containing the pernicious anemia factor have been shown to contain factors which can be measured on the basis of a growth response in chickens and in rats (Betheil et al., '47; Nichol et al., '47). Between 2 and 6 weeks are required to show activity in these animals, whereas a conditioning period of approximately 3–6 months in addition to a 1–2 month test period are required for the dog. Consequently, the rat and chicken appear much more practical for the assay than the dog, providing that all three animals are responding to the same factor. However, the dog assay is still important because of the hematological changes observed. Whether or not the same factor is measured in each of the three species is still uncertain.

SUMMARY

Young growing dogs were fed a niacin-deficient purified ration containing 1% sulfasuxidine. When the animals developed symptoms of blacktongue, they were given single doses of niacin. This therapy was found to be only partially suc-

cessful in combating the loss in weight. Folic acid was found to play an important part in bringing about more consistent responses to niacin, but it had no apparent effect on the macrocytic anemia which developed progressively. Liver extracts rich in the pernicious anemia factor were effective in restoring the blood picture and general health of the animals. These extracts were only partially effective when given alone, but in combination with folic acid gave complete recovery. When higher levels of protein were fed, however (24–30%), the need for folic acid could not be shown. As little as one U.S.P. unit of reticulogen per day is sufficient to bring about complete recovery. Apparently the factor is not stored to any great degree, and must be injected regularly.

ACKNOWLEDGMENTS

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VITAMINS REQUIRED BY SWINE FOR GROWTH, WITH SOME OBSERVATIONS ON REPRODUCTION

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The earlier literature on the adequacy of synthetic diets for pigs was reviewed by McRoberts and Hogan ('44) and requires no further comment at this time. These investigators reported that in their experience synthetic diets were inadequate for pigs. The mortality rate was high and the animals usually grew slowly, though occasionally a pig on an artificial diet would grow at almost a normal rate. The basal diet used most extensively contained thiamine, riboflavin, pyridoxine, pantothenic acid, nicotinic acid, choline, and vitamins A, D, E, and K. The diet was not improved by the addition of ascorbic acid, biotin, inositol, and *p*-aminobenzoic acid, but it became entirely adequate for growth when a water extract of liver was included. This was regarded as evidence that the pig requires a vitamin then unrecognized.

Several groups of investigators, including Wintrobe, Stein, Follis and Humphreys ('45); Lindley and Cunha ('46); Cunha, Bustad, Ham, Cordy, McCulloch, Woods, Conner, and McGregor ('47); and Powick, Ellis, Madsen, and Dale ('47) attained a considerable degree of success in rearing pigs on synthetic diets. However, most of their experimental animals

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The data in this manuscript were taken from a thesis to be submitted by Mr. Anderson in partial fulfillment of the requirements for the A.M. degree.

were at least three weeks old at the beginning of the experimental period. Wintrobe and collaborators used a few that were 16 to 22 days old when first given a synthetic diet. One of the most successful vitamin mixtures used by this group did not contain niacin, inositol, or *p*-aminobenzoic acid. Lindley and Cunha ('46) obtained no evidence that either biotin or inositol should be included in the diet of the pig. Cunha et al. ('47) reported that their ration was not improved by the addition of folic acid and *p*-aminobenzoic acid, either alone or in combination with biotin and inositol. Powick et al. ('47) concluded that between the ages of three and 9 weeks the pig requires between 0.6 and 1.0 mg daily of nicotinic acid per kilo of live weight.

Cartwright, Wintrobe and Humphreys ('46) placed a pig at the age of 30 days on a synthetic diet which included 2% of sulfasuxidine. The pig grew slowly and developed symptoms of a nutritional deficiency. After 140 days a normocytic anemia was well established. The animal was given intramuscular injections of a commercial liver extract, presumably prepared for the treatment of pernicious anemia, and recovered. Four other pigs received this same diet, except that crude casein replaced the vitamin-free product; they were normal in appearance and their growth rates were not retarded.

Welch, Heinle, Sharpe, George and Epstein ('47) also included succinylsulfathiazole in a synthetic diet they supplied to pigs, but no evidence was obtained that it retarded the rate of growth or interfered with the production of erythrocytes or hemoglobin. After 16 days one of the pigs in the group which did not receive pteroylglutamic acid was given a chemical antagonist of that vitamin. The pig grew slowly, became unthrifty in appearance and became anemic. When the condition of the pig became critical, the vitamin-free casein in the diet was replaced by crude casein, and in addition an alcoholic extract of crude casein and human gastric juice were given together by stomach tube. After 10 days of this supplementation, the pig was returned to the original diet and

apparently became entirely normal. It is not clear why the purified diet failed to interfere with growth and hematopoiesis as described by Cartwright et al. ('46) but the difference in the vitamins supplied may have had some effect on the results. The observations of Cartwright and collaborators ('46) and of Welch et al. ('47) give some support to the conclusion of McRoberts and Hogan ('44) that the pig requires an unrecognized vitamin.

Russell, Teeri and Unna ('48) began with pigs that were 28–31 days old and maintained them as long as 469 days on a synthetic diet. The vitamin mixture did not include biotin, pteroylglutamic acid, or vitamin K. The pigs grew normally and were normal in appearance but neither the males nor females were able to reproduce. Ensminger, Bowland and Cunha ('47) observed that sows which consume synthetic diets over a long period are unable to rear their litters. Other evidences of the inadequacy of the ration were difficulty in getting the sows to conceive and abnormalities in the pigs, such as weakness at birth and a high mortality rate, hernia, kinked tails, enlarged forelegs, and abnormal liver and kidneys.

During the period since the 1944 paper of McRoberts and Hogan appeared, biotin has been supplied in larger amounts and synthetic pteroylglutamic acid has become available. The object of the present investigation has been to test the adequacy for pigs of synthetic diets containing these two vitamins, in addition to the others mentioned, from the beginning of the experimental period.

EXPERIMENTAL

The experimental procedure used has been described by McRoberts and Hogan ('44) and only its more essential features will be repeated. The new-born pigs were left with the dam for two days, as in our earlier experience the mortality rate had been high even on diets believed to be adequate if the pigs failed to receive colostrum. The diets, described in table 1, were synthetic in type but homogenized in water to

reduce the content of solids to 19%. The pigs were hand-fed from a bottle 8 times daily, at three-hour intervals, during the first two weeks. During the next 6 weeks they were fed 7 times daily, beginning at 6:00 A.M. and ending at 12:00 midnight. If kept on experiment for a longer time, the pigs were then given dry feed. Since it is essential to keep young pigs warm, they were quartered in a room held at a temperature of 75°F. or higher.

TABLE 1

Composition of experimental diets

BASAL MIXTURE				SUPPLEMENTS			
Ingredient	Diet number			Vitamins[1] per 100 gm of diet (all diets)			
	327	330	335				
	%	%	%				
Casein (Labco)	30	15	30	Vitamin A (I.U.)	2000	Pyridoxine-HCl (mg)	1
Sucrose	30	70	30	Vitamin D (I.U.)	400	Ca-pantothenate (mg)	3
Corn starch	5	..	5	Vitamin E (mg)	4	Nicotinic acid (mg)	4
Wood pulp	..	5	5	Vitamin K (mg)	2	Choline-Cl (mg)	100
Lard	30	5	25	Thiamine-Cl-HCl (mg)	1	Inositol (mg)	100
Salt mixture[2]	5	5	5	Riboflavin (mg)	1	Biotin (mg)	0.03
						Pteroylglutamic acid (mg)	0.2

[1] Vitamins A and D were purchased as a concentrate from Mead Johnson and Company, Evansville, Indiana. The pteroylglutamic acid was generously supplied by Dr. T. H. Jukes, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York. All other vitamins were generously supplied by Dr. D. F. Green of Merck and Company, Inc., Rahway, New Jersey.

[2] Richardson and Hogan ('46).

Three separate tests of the adequacy of artificial diets were carried out. The 6 purebred Chester White pigs used in the first trial were kept on cement floors with shavings as bedding. Because of uncertainty as to the adequacy of the diet, three of the 6 received a ration which was similar to Diet 327 except that a liver extract was substituted for an equal weight of sucrose. During the first 23 days the modified ration contained 2% of a pork liver extract fraction. However, the fraction did not seem to improve the ration in any respect, and on the 24th day 5% of a water extract of pork

liver was included. Since it is doubtful that the extract had any effect on the response of the pigs, no special comment on it is required.

The other three pigs received Ration 327 with no modifications, and will be treated in more detail. As soon as they were transferred to the artificial milk they developed a diarrhea which was most marked during the first two weeks but which persisted in some degree throughout the experimental period. These symptoms were also characteristic of the two other trials which were carried out later on. It seemed impossible when the diarrhea first appeared that there could have been time for a nutritional deficiency to develop, and it seems probable now that one or more constituents of the diet is mildly irritating to the intestinal tract of a young pig. Possibly the irritation could be avoided by reducing the amount of mineral salts or by replacing sucrose with a simple sugar.

During the first three weeks the pigs grew at only a moderate rate and there was a definite, though slight, brownish exudate around the eyes of each pig, which later disappeared. After the third week the pigs had tremendous appetites and grew more rapidly than did their litter mates which had been left with the sow. It happened not infrequently that the pigs would regurgitate a small quantity of food, but this seemed to be due to excessive consumption rather than to a nutritional deficiency. Normally, young pigs feed about every two hours, or less. It was impracticable to feed the experimental diets that often, and for that reason the food intake per feeding was enormous. However, the pigs were altogether normal in appearance at the end of the experimental period and there was no definite evidence that the diets were deficient in any respect. The growth rates in all three trials are shown in table 2.

The response of the pigs in this first trial, both in rate of gain and in appearance, exceeded our expectations and was repeated with some variations in later tests.

TABLE 2

Growth of pigs on synthetic diets

NO. AND SEX	BODY WEIGHT		
	At birth	2 days old	56 days old
	lbs.	*lbs.*	*lbs.*
Trial 1, Chester Whites			
Ration 327			
38 F	3.5	4.1	40.2
15 M	2.7	3.7	35.6
19 M	2.6	3.1	32.7
Avg.	2.9	3.6	36.2
Ration 327 modified to contain liver extract:			
14 M	3.2	4.1	55.5
16 M	2.7	3.7	39.0
13 M	2.5	3.2	36.6
Avg.	2.8	3.7	43.7
Trial 2, Poland Chinas			
Ration 327, contains pteroylglutamic acid:			
4 F	3.2	3.7	27.8
3 F	3.0	3.4	21.6
1 F	3.0	3.5	22.8
Avg.	3.1	3.5	24.1
Ration 327, does not contain pteroylglutamic acid:			
8 F	2.6	3.5	18.5
1 M	3.0	3.5	18.7
5 F	2.7	3.3	25.0
Avg.	2.8	3.4	20.7
Trial 3, Duroc Jerseys			
Ration 327, contains pteroylglutamic acid:			
On screen floors			
61 F	3.6	3.9	29.5
58 M	3.1	4.0	29.3
Avg.	3.4	4.0	29.4
On cement floors			
60 F	3.8	4.0	34.0
55 M	2.2	3.5	17.0
Avg.	3.0	3.8	25.5
Ration 334, does not contain pteroylglutamic acid:			
On screen floors			
64 F	3.6	3.7	23.5
59 M	3.4	4.1	35.2
Avg.	3.5	3.9	29.9
On cement floors			
56 F	2.9	3.6	36.0
60 M	4.4	4.3	33.6
Avg.	3.7	4.0	34.8
Fortified cow's milk:			
On cement floors			
57 F	2.7	3.4	56.5
63 M	4.4	4.9	54.0
Avg.	3.6	4.2	55.3

In the next trial the pigs were purebred Spotted Poland Chinas. They were on floors of wide mesh screen, in order to reduce the possibility of coprophagy. An attempt was also made in this trial to determine whether pteroylglutamic acid is required by the pig and the animals were, therefore, divided into two groups. One received Ration 327 and the other Ration 334, which does not contain pteroylglutamic acid but is identical with Ration 327 in all other respects.

The outcome of the second trial was less encouraging than that of the first. The diarrhea was not more pronounced than in the first trial and the pigs were normal in appearance, but the rate of growth was disappointing. The explanation of the difference is not apparent, but several possibilities have been considered: (1) The pigs may have been of a slow-growing strain. (2) The wire screens may have been responsible for the retardation by reducing the practice of coprophagy. (3) The trial was conducted in the winter months and during that season the heating system was unable to keep the room as warm as was desired. The pigs which received pteroylglutamic acid grew more rapidly than those which did not receive it, but comparison with trial 3 suggests that the difference was fortuitous.

There were 10 pigs in all in the third trial. Two were used as positive controls to establish the growth rate on an adequate diet. These two pigs received fortified cow's milk (1 liter of milk plus 60 gm sucrose, 2.5 gm ferrous sulphate, 0.2 gm cupric sulphate, 0.2 gm manganous sulphate, 0.02 gm potassium iodide), and were on cement floors with shavings. There were 8 experimental pigs; 4 were on floors of wire screen and 4 on cement floors with shavings for bedding. This arrangement should show whether or not coprophagy had any relation to the nutritional status of the pigs. Two of each group received Ration 327 and two received Ration 334, in order to obtain additional data on the requirement of the pig for pteroylglutamic acid. The pigs were purebred Duroc Jerseys. They did not grow as rapidly as did those in the first trial, but more rapidly than those in the second.

There were intermittent attacks of diarrhea, but the pigs had the appearance of health and thrift and seemed to be normal in every respect. Insufficient data are available for a final decision, but the evidence indicates that when pigs consume artificial, so-called "synthetic" diets, they grow more rapidly when on solid floors. It is difficult to eliminate all possibility of access to feces, but it seems quite certain that pigs can attain an average rate of growth without practicing coprophagy. The pigs not receiving pteroylglutamic acid grew as rapidly as did those which did, and we have no reason now to suppose that this vitamin is a dietary essential for the pig.

The only animal which calls for special comment is 55M, which was quartered on the cement floor and received pteroylglutamic acid. From the beginning this animal seemed eager for food but would never consume at any one time as much as the others did. The pig seemed definitely abnormal in some way, but there was no specific symptom which suggested that the abnormality was due to inadequacy of the ration. When 60 days of age, with a weight of 19.8 pounds, this pig was changed to the diet of fortified cow's milk, which was supplied in an open container so the milk was available practically all the time. At the end of 25 days the pig weighed 43 pounds. While consuming the synthetic diet the average daily gain was 0.23 pounds, and after the change to fortified cow's milk, the average daily gain was 0.93 pounds, a normal rate for that age. When 85 days old, this pig was given a practical ration but it consumed little feed and did not gain in weight. The animal was sacrificed for post-mortem examination [2] but the only abnormalities observed were inflammation in restricted areas of the colon and petechial areas in the stomach with some deep-seated fibrosis, which were presumably the consequence of a previous low-grade inflammation. It is impossible to decide whether or not the diet of the pig was responsible for its lack of thrift.

The pigs which consumed cow's milk grew much more rapidly than did those fed the "synthetic" milk, and we have

[2] Courtesy of J. E. Weinman, D.V.M.

adopted the working hypothesis that the artificial diet is deficient in a nutrient essential for maximum growth and for the optimum nutritional state.

During the first trial, in the fall of 1946, there was one female pig on Ration 327 and since this group had exceeded all expectations in appearance and performance, an attempt was made to continue her on an artificial diet through a reproductive period. When she was 87 days old, her ration was modified somewhat to reduce the protein content. The composition of this ration, no. 330, is shown in table 1. This gilt, no. 38, continued to grow at a tremendous rate, and eventually her feed intake was limited to prevent her from becoming overfat. She conceived on March 12, and farrowed on July 7, with no untoward incidents. She gave birth to 11 pigs that were alive and two that were dead. One of the dead pigs had died at least two or three weeks before parturition; the other was smothered in the chorionic membrane. The appearance of the pigs would indicate that the milk of this sow was adequate in quality, but never adequate in quantity. Five of the mammary glands were inflamed and nonfunctional, and only 5 of the others secreted significant quantities of milk. There was delay in the initiation of milk secretion and at the beginning of the second week only 5 pigs were alive. During the first 10 days of lactation the feed intake was low and therefore the ration was changed on the 11th day to no. 335, table 1. After that time the feed intake increased and apparently the milk flow increased also. When the litter was about 5 weeks old the rate of feed consumption declined again, with a parallel decrease in the rate of milk secretion, and it was also noted that the sow was severely anemic. In view of the report of Cartwright, Wintrobe, and Humphreys ('46), the sow was given injections of 2 ml (30 units) of liver extract[3] for 14 successive days, but the treatment was ineffective. Whether it was given too late, or whether it did not contain the nutrients she required, we are unable to say. She died on the 56th day

[3] Eli Lilly Co., Indianapolis, Indiana.

TABLE 3

Abbreviated history of the sow reared on an artificial, so-called "synthetic" diet

AGE	WEIGHT	DAILY GAIN IN WEIGHT	DAILY FEED INTAKE	ERYTHROCYTE COUNT
days	*lb.*	*lb.*	*lb.*	*million/mm³*
Ration 327				
56	40			7.485
86	63	0.76	1.6	
Ration 330				
116	119	1.86	3.8	7.625
146	182	2.10	6.6	
176	246	2.13	6.8	
206	306	2.00	6.3	
236	352	1.53	4.5	7.526
266	410	1.93	5.1	
294	465	1.96	6.1	
		Farrowed on 294th day		
295	419		0.0	
306	369		0.5	7.724
310	369		4.0	
Ration 335				
320	352		6.0	
330	348		8.0	
334	338		7.0	3.040
337	330		4.0	1.225
339	327		1.0	2.375
346				1.870
350 died				

after farrowing.[4] A brief history of this animal is shown in table 3.

When it became evident that sow 38 was anemic her 5 remaining pigs were removed, and reared on the same diet as their mother had received. The average weight at 56 days was 24 pounds, with a range of 16.7 to 34.5 pounds. This was a

[4] Pathologist's report on sow 38 (courtesy of M. P. Neal, M.D.): *Bone marrow*: no recognizable hematopoietic tissue; functional marrow replaced by non-functional fat; *liver*: remarkably pronounced interlobular connective tissue; degeneration and necrosis of liver cells beginning around the central veins and extending through the central zone area; *kidneys*: extensive albuminous degeneration and hydropic infiltration of the proximal tubules; *spleen*: a loss of Malpighian corpuscles and of parenchymatous elements and a deposit of fibrinous material in a tissue closely resembling hematopoietic structures.

remarkable recovery, and indicates that the diet could not be seriously inadequate for growth.

DISCUSSION

It is clear that our efforts to rear pigs on synthetic diets were much more successful than those of McRoberts and Hogan ('44). The reason is uncertain, but some of the possibilities will be mentioned. The salt mixtures used in the two trials were not the same, but neither the qualitative nor quantitative differences seemed large enough to be significant. There were minor differences in the amounts of the vitamins included in the diets, but according to present knowledge the least amount of any of the vitamins supplied by McRoberts and Hogan was more than enough. It seems improbable that pteroylglutamic acid contributed to our greater success in these recent trials, because the pigs grew as well without it as they did with it. Biotin and inositol were always included in the rations described in this report but our experience, and that of other investigators mentioned, leaves it uncertain whether either of these vitamins contributed to the improvement in the diet. The average growth rate of the experimental pigs was equal to that of suckling pigs in commercial production, but the high degree of variability requires some comment. The variable growth rates may be partially explained by genetic differences, but it seems more probable that the experimental diet was inadequate in some degree. This conclusion is supported by the poor appetite of sow 38 after farrowing, her failure in milk secretion, her severe anemia, the extreme pathological condition of some of her internal organs, and her early death. However, the possibility that some or all of these abnormalities were the consequences of mastitis may require consideration.

SUMMARY

1. Seventeen pigs were reared on a synthetic diet from the time they were two days old until they were 56 days, or more, of age. There were no mortalities, though all of the pigs were

subject to intermittent attacks of diarrhea. The rate of growth was variable but the average weight at 56 days was as high as would be expected according to current growth standards. With one exception, all of the pigs were normal in appearance.

2. One female pig was retained on the synthetic diet through the reproductive stage. She bore a normal litter but her flow of milk was scanty. She became severely anemic while lactating, and died. On autopsy an extreme pathological condition was discovered in the bone marrow, liver, kidneys and spleen.

3. These findings indicate that the artificial, so-called "synthetic" diet used in this study is slightly inadequate for growth and seriously inadequate for lactation.

ACKNOWLEDGMENTS

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SOUTHERN PEAS AND OTHER LEGUME SEEDS AS A SOURCE OF PROTEIN FOR THE GROWTH OF RATS

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THREE FIGURES

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Southern peas (edible varieties of the cowpea, *Vigna Sinensis,* Brittingham, '46), of which the most generally consumed variety is the Blackeye pea, are an important source of food for a large section of the population throughout the South. Comparatively few studies have been carried out on the nutritive value of varieties of Southern peas, and since they are used widely as a source of human food it is desirable to have more information about their relative value as a source of protein for growth.

Osborne, Mendel and Ferry ('12) found the isolated globulin, virginin — the chief protein of the cowpea — to have a slightly greater nutritive value than wheat gliadin. Finks, Jones and Johns ('22) in a study of the field varieties, Groit and Brabham, obtained a growth rate in rats of ⅓ to ⅔ of normal with either raw or cooked cowpea meal. These same authors found that the addition of 0.33% cystine to the cooked meal gave a normal rate of growth, but the addition of the cystine to the raw meal gave no better growth than the raw meal alone.

An increase in the nutritive value of soybean protein on heating has been demonstrated by several investigators, and

these studies have been reviewed recently by Evans and McGinnis ('46). Finks and Johns ('21) reported that rats fed raw Lima beans, *Phaseolus lunatus,* failed to gain weight even when the protein was supplemented with 0.3% of cystine. The cooked beans were no better than the raw beans unless supplemented with cystine. When 0.3% of cystine was added to the cooked beans, the rats grew at a normal rate.

Since cystine is not an indispensable amino acid, the response to the addition of cystine in these earlier experiments requires some explanation. Rose ('38) has shown that cystine stimulates growth when methionine is supplied in sub-optimal quantities, but not when it is entirely absent from the diet. The diets which were used in these earlier experiments must have contained a sub-optimal amount of methionine, and therefore growth was stimulated when cystine was added.

Everson and Heckert ('44) tested the nutritive value of several legumes for the growth of rats, both in the raw state and after heating in a pressure cooker at 15 pounds for 15 minutes. The rats which received raw kidney beans, navy beans or pinto beans lost weight and died within 3 or 4 weeks. Those which received raw Lima beans lost weight but survived the 8-week experimental period. The nutritive value of all the legumes was increased by heating with the exception of peas in which it was decreased slightly. Woods, Beeson and Bolin ('43) and Lehrer, Woods and Beeson ('47), in a study of the nutritive value of Alaska field peas, reported that the daily gain was tripled and the protein efficiency doubled when either the cooked or raw peas were supplemented with 0.3% of methionine. Heating the peas dry for 1½ hours at 140°C. or autoclaving for 1½ hours at 17 pounds pressure decreased the nutritive value, and this decrease was attributed to the effect of heat on the protein. Russell et al. ('46) reported the results of their studies on several varieties each of Lima beans, snap beans *(Phaseolus vulgaris)* and English peas. The legumes were soaked overnight and cooked in the soaking water in a procedure similar to that used for human consumption. Growth was poor in all cases unless the legumes

were supplemented with 0.1% of methionine. Raising the level of methionine to 0.6% gave a further growth response except for three varieties of peas, in which cases a loss in weight occurred.

Ham and Sandstedt ('44) and Ham et al. ('45) reported the presence of a trypsin inhibitor in soybean meal, and Bowman ('44) found the trypsin inhibitor in navy beans as well as soybeans. Kunitz ('45, '46, '47) has isolated the crystalline trypsin inhibitor from soybeans and has studied its properties. This inhibitor is destroyed by heat. It is probably present in certain other legume seeds, but available data indicate that it is not present to any appreciable extent in the various varieties of Southern and English peas which were used in this study.

Observations on the comparative nutritive values of Southern peas *(Vigna Sinensis)*, Lima beans *(Phaseolus lunatus)*, pinto beans *(Phaseolus vulgaris)*, English peas *(Pisum sativum)*, casein and egg albumin are given in this report.

EXPERIMENTAL

Albino rats 28 days old and weighing 35 to 45 gm were given the experimental diets for a period of 4 weeks. Litter mates were matched as to sex and weight and distributed as far as possible among the groups which received the different legumes. They were kept in individual cages and food and water were supplied ad libitum except in the paired feeding trials. Two separate groups, consisting of two males and two females each, were used in every test and the two tests were run at different times. Each curve in the charts represents the combined average gain in weight of the 8 rats used in the test.

The lugumes were purchased from a seed supply house or grown on the Texas A. and M. Horticultural Farm. The nutritive value of both the raw and heated legumes was studied. Before heating, 1 kilo of the ground seed was mixed with 1500 ml of distilled water and allowed to stand for one to two hours. The mixture was then heated in the autoclave for 30 minutes at 15 pounds pressure and dried in an air oven at 60°C. The

legumes were added to the diet at a level equivalent to 10% protein (N × 6.25) in all cases except when comparison was made of casein with Southern peas by the ad libitum and paired feeding methods; the amounts provided in these tests are given in figure 1. The casein and egg albumin were added at the levels shown in figures 1 and 3. The composition of a typical diet which supplies 10% protein is given in table 1.

Ad libitum and paired feeding methods

A preliminary test was carried out to determine whether or not the ad libitum method of feeding would be a satisfactory

TABLE 1

Composition of a typical diet

BASAL MIXTURE	%	VITAMIN SUPPLEMENTS PER 100 GM OF BASAL MIXTURE	
Legume (equivalent to 10% protein)	44		*mg*
Cerelose	38	Menadione	2.5
Wood pulp	3	Alpha tocopherol	2.5
Mineral mixture [1]	5	Thiamine hydrochloride	0.4
Lard	10	Riboflavin	0.4
VITAMIN SUPPLEMENTS PER 100 GM OF BASAL MIXTURE		Pyridoxine	0.4
		Calcium pantothenate	1.0
		Niacin	5.0
Vitamin A	3,000 I.U.[2]	Choline chloride	100.0
Vitamin D	425 I.U.	Biotin	0.01

[1] Richardson and Hogan, '46.

[2] Vitamins A and D were supplied by Mead Johnson's Oleum Percomorphum.

means of investigating the relative value of two proteins if they were fed at the same protein level. In this test Southern peas were compared with casein by both the ad libitum and the paired feeding methods. Both Southern peas and the casein were supplied at protein levels of 12 and 15%. These data are summarized in figure 1. The difference in weight gains are practically the same regardless of whether the rats were fed by the paired feeding or the ad libitum method.

This observation shows that the calculation of protein efficiency does not give any additional information in regard to

the relative value of the two proteins. Hegsted and Worcester ('47) have concluded, from a study of the relation between protein efficiency and gain in weight on diets of constant protein content, that gain in weight alone and protein efficiency alone are equally accurate in measuring the relative value of proteins. In view of these observations, the rats in all subsequent experiments were fed ad libitum and the curves in the charts represent the average gains in weight obtained by this method.

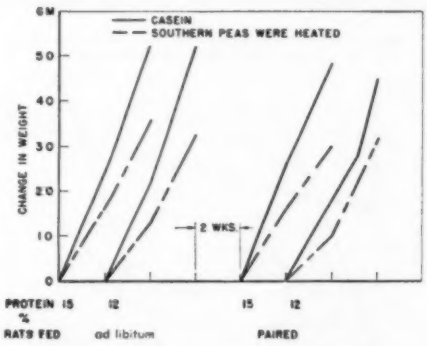

Fig. 1 Comparison of the average gain in weight of rats when they were fed Southern peas and casein by the ad libitum and by the paired feeding methods.

Amounts of methionine required

The effect of supplementing heated pinto beans and Southern peas with two levels of methionine is summarized in figure 2. Either legume supports a very slow rate of growth when it is fed as the sole source of protein in the diet, but when supplemented with 0.2% methionine practically normal growth is obtained. Two-tenths % of methionine gave a maximum rate of growth. One-tenth % was not enough, and in the few trials where 0.3% was fed the gains were no faster than they were with 0.2%.

Comparison of legumes, legumes plus methionine, casein and egg albumin as sources of protein

Southern peas were compared with Lima beans, pinto beans, English peas, casein and egg albumin as sources of protein for the growth of rats. The results are summarized in figure 3.

Rats which received raw Southern or raw English peas grew slowly, but they grew at a slightly faster rate than those which received the heated peas. When the English peas were supplemented with 0.2% methionine the rats grew at a normal rate, but again those receiving raw peas grew at a slightly faster rate than those receiving heated peas. A slight de-

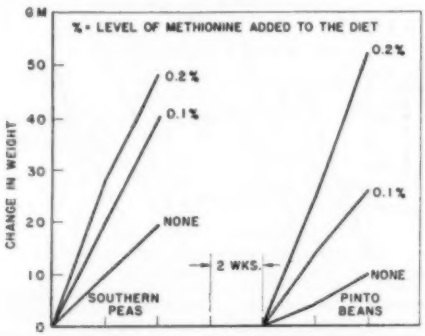

Fig. 2 The amount of methionine required to give a maximum gain in weight in rats when all the protein in the diet is supplied by pinto beans or Southern peas.

crease in the nutritive value of peas when autoclaved or heated dry has been reported also by Everson and Heckert ('44) and by Lehrer et al. ('47). Supplementing the heated Southern peas with 0.2% methionine increased their nutritive value, but the average gain was less than it was with egg albumin or with the other legumes plus methionine.

The results obtained with raw Lima and raw pinto beans differed from those which were obtained with either of the raw peas. The rats which received the raw beans lost weight and two receiving raw Lima beans died on the 23rd and 26th day, respectively. The weight for this group for the 4th week in

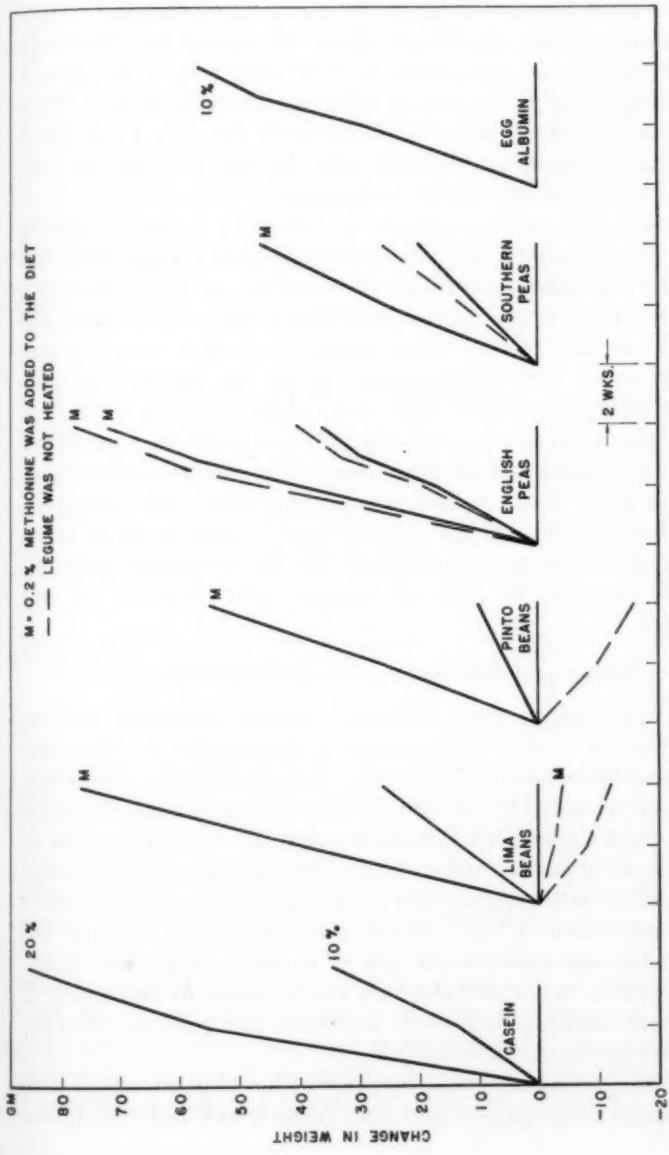

Fig. 3 Comparison of casein, Lima beans, pinto beans, English peas, Southern peas and egg albumin as sources of protein for the growth of rats. The varieties of legumes were as follows: Lima bean, Henderson bush; pinto bean, comercial; English peas, Little Marvel; Southern peas, Dwarf California Blackeye no. 5.

figure 3 represents the average of 6 rats. The addition of 0.2% methionine to the raw Lima beans decreased the rate loss slightly, but these beans were a very poor source of protein for the growth of rats even with methionine added. This suggests that the low nutritive value of the raw Lima bean is probably due to the presence of a trypsin inhibitor as well as an inadequate amount of methionine.

English peas were superior in nutritive value to heated Lima beans and to raw or heated Southern peas, and the heated Lima beans were slightly superior to Southern peas. The protein of heated pinto beans had a lower value than the protein of any of the other legumes studied until it was supplemented with methionine. When the heated legumes were supplemented with 0.2% methionine the rats grew at a rapid rate in every case, but the greatest gains were obtained with Lima beans and English peas. The rats receiving these legumes grew faster in both cases than those which received egg albumin. Pinto beans plus methionine supported as rapid a rate of growth as egg albumin but the Southern peas, although superior to 10% of casein, were inferior to egg albumin.

Amino acid deficiency of Southern pea protein

The data which were discussed in the preceding section show that in Southern pea protein methionine is either low or unavailable, and suggest that there is either a deficiency or a low availability of one or more other essential amino acids. Schweigert ('48) has shown that all the tryptophane in soybean oil meals is not available for the growth of chicks, and Kuiken and Lyman ('48) have reported that the lysine in cottonseed flours is only 64.0% available, while some of the other essential amino acids are 95 to 100% available. Tests were carried out with these two amino acids to determine if they were limiting factors in Southern pea protein. The results are summarized in table 2.

The rats which received Southern peas supplemented with methionine alone gained less than those which received lysine

and tryptophane in addition to methionine. A statistical analysis showed that these differences are highly significant. The gains of the rats which received Southern peas supplemented with the three amino acids did not differ significantly from those of the animals receiving egg albumin. It may be concluded from these data that Southern pea protein supplies an inadequate amount of lysine or tryptophane or both for the normal growth of rats. It has not been demonstrated whether the deficiency is due to a low amino acid content or to a low availability of the amino acid or acids. Lysine and tryptophane were not tested separately.

TABLE 2

Amino acids as supplements to Southern pea protein for the growth of rats

SOURCE OF PROTEIN	AMINO ACID SUPPLEMENT		RATS	AVERAGE GAIN IN WEIGHT [1]
	%		no.	gm
Southern peas	Methionine	0.2	10	44
Southern peas	Methionine Lysine Tryptophane	0.2 0.5 0.1	12	57
Egg albumin	None		12	56

[1] Experimental period 4 weeks.

Comparison of three varieties of Southern peas

The three varieties of Southern peas which are generally grown for human consumption in Texas are Long Pod Cream, Dwarf California Blackeye no. 5 and Jackson Purple Hull. These varieties were compared as sources of protein for the growth of rats at a level equivalent to 10% of protein. The results are summarized in table 3. Each variety was autoclaved and tested with and without methionine. The rats gained more in each case when methionine was added. The Long Pod Cream and California Blackeye no. 5 varieties were about equal in value when they were supplied at the same level of protein. However, it requires a total of 42% of the California no. 5 peas as compared to 37.5% of the Long Pod

Cream peas to give the same level of protein in the diet. The rats which received Jackson Purple Hull peas gained an average of 12 gm without and 32 gm with methionine; those which received Dwarf California Blackeye no. 5 and Long Pod Cream each gained an average of 20 gm without methionine and 45 gm with it. A statistical analysis showed that the differences in the gains of the rats which received the Jackson Purple Hull peas and those which received the other two varieties were highly significant, and it may be concluded that these peas have a lower nutritive value than the other two varieties when fed either with or without methionine. The data show that the protein of the Jackson Purple Hull variety

TABLE 3

Comparison of the nutritive value of the protein of 3 varieties of Southern peas

VARIETY	PROTEIN CONTENT	PEAS REQUIRED TO GIVE 10% PROTEIN	AVERAGE GAIN IN WEIGHT [1]	
			Methionine	
			None	0.2%
	%	%	*gm*	*gm*
Jackson Purple Hull	27.0	37.0	12	32
Dwarf Calif. B. no. 5	23.8	42.0	21	45
Long Pod Cream	26.7	37.5	20	45

[1] Experimental period 4 weeks. There were 2 trials in each test consisting of 6 rats each. The average gain in weight is the average of 12 rats in each case.

differs from that of the other varieties either in amount or in the availability of some other indispensable amino acid or acids.

SUMMARY

Lima beans, pinto peas, English peas and Southern peas were compared with casein and with egg albumin as sources of protein for the growth of rats. The legumes were tested raw and after they had been autoclaved at 15 pounds pressure for 30 minutes. The heated Lima and pinto beans were superior to the raw beans as sources of protein; the raw Southern and English peas were slightly superior to the heated peas.

The nutritive values of all the legumes were increased by the addition of 0.2% of methionine if the legume was heated before the methionine was added. Raw Lima beans were improved only slightly by the addition of methionine. On the other hand, raw English peas were improved markedly by supplementation with this amino acid, and the raw peas plus methionine were slightly superior to egg albumin when the heated legumes were supplemented with 0.2% of methionine. Southern peas were superior to casein but they had a lower value than egg albumin unless lysine and trptophane were fed in addition to methionine.

In tests with Southern peas and with pinto beans 0.2% of methionine was required to give a maximum rate of growth. One-tenth % was not enough and 0.3% was no better than 0.2%.

The Jackson Purple Hull, Long Pod Cream and Dwarf California Blackeye no. 5 varieties of Southern peas were compared with each other as sources of protein for growth. The tests were carried out in each case with heated peas and with and without the addition of methionine. The Jackson Purple Hull had a lower nutritive value in both tests than the other two varieties. These data suggest that protein availability may be lower in the Jackson Purple Hull, or that a smaller quantity of one or more of the essential amino acids may be present.

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THE OCCURRENCE OF 5 B-VITAMINS IN THE TISSUES OF PREGNANT RATS FED RATIONS SATISFACTORY AND UNSATISFACTORY FOR REPRODUCTION [1]

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Unsuccessful reproduction in rats, as evidenced by the incidence of resorptions, the development on the day of parturition of a syndrome resembling toxemia of pregnancy, and the production of many non-viable young, has been observed in animals fed a ration containing partially dried autoclaved pork muscle and yeast as the principal sources of the B-vitamins and of protein (Swanson et al., '43; Armstrong and Swanson, '43). The toxemia characteristic of animals fed this experimental ration developed suddenly; the rats lost all muscle tone, the hair was erect, the feet and ears were very pale and the entire body felt cold. Hematuria and vaginal hemorrhage frequently were present. Death was preceded by convulsions or coma. In most cases the female died before parturition occurred, although in some instances the young were born dead. The syndrome of toxemia occur-

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red sporadically, varying from a maximum incidence of 43% of the animals during initial studies to less than 5% during certain years. The number of pregnancies terminating in resorptions varied from zero to 11% in the earlier studies. More recently the ratio has been somewhat greater.

For the past several years this ration has been studied in an effort to determine the nature of the dietary deficiency responsible for the poor reproductive performance. The diet was supplemented in turn with various materials, some natural and others synthetic. Fresh beef liver alone afforded protection against the reproductive disorders. Beef muscle, when substituted for the pork in twice the quantity in which it occurred in the original diet also improved the reproductive record. In general, however, little conclusive evidence was obtained as to the dietary causes of unsuccessful reproduction, and a new method of attack was necessary.

It seemed that if the problem could be approached from the standpoint of examining vitamin depositions in tissues both of normal animals and rats fed the experimental pork diet, information might be gained regarding the nature of the dietary inadequacy. The facts (a) that liver exerted a protective influence, and (b) that the recent occasional appearance of the toxemia occurred when yeasts containing higher quantities of certain of the B vitamins were used, led us to believe that a deficiency, either acute or border-line, of one or more of the B-vitamins might be the causative factor.

The present study reports data on the occurrence of thiamine, riboflavin, niacin, pantothenic acid and biotin in the hepatic, carcass, and fetal tissues of experimental and control groups of rats at the termination of the first and second pregnancies. One group received the original pork-containing ration designated as Pork I and the second, a modification of the Steenbock stock diet (Steenbock XV) which has been used successfully in this laboratory for many years for the production of stock animals.

EXPERIMENTAL

Animals

Seventy-two female rats of inbred Wistar stock were used in the experiment. All animals received a modification of the stock ration originally formulated by Steenbock ('23) until the time of the opening of the vaginal orifice. The distribution of the experimental animals is given in table 1. When animals receiving the Pork I ration developed the syndrome of toxemia, they were studied as a separate group. Replacements were made from stock so that a total of 10 females comprised each main experimental group.

TABLE 1

Distribution of experimental animals

GROUP	DIET	NUMBER OF ANIMALS	TIME AT WHICH ANIMALS WERE SACRIFICED
1	Steenbock	10	Day of opening of vaginal orifice
2a	Steenbock XV	10	28 days (± 3 days) after opening of vaginal orifice
2b	Pork I	10	28 days (± 3 days) after opening of vaginal orifice
3a	Steenbock XV	10	21.5 days (± 4 hours) after 1st mating
3b	Pork I	10	21.5 days (± 4 hours) after 1st mating
4a	Steenbock XV	10	21.5 days (± 4 hours) after 2nd mating
4b	Pork I	10	21.5 days (± 4 hours) after 2nd mating

Four animals which developed toxemia were available for assay. Two of these females were originally in group 4b, and two were taken from other experiments. The latter animals had been receiving a ration similar to Pork I except that the amount of yeast had been reduced from 5 to three parts. The syndrome of toxic pregnancy appeared earlier in the gestation period during this study than was the case in previous investigations; 3 of the 4 animals showed pronounced signs of toxemia on the 18th day of pregnancy and one on the 16th day. Tissues were taken for analysis directly following the death of one animal. The other three were in a succumbant state.

Diets

Both rations were offered ad libitum and records of the daily food consumption were kept. The Pork I ration contained the following ingredients: partially dehydrated pork muscle, 25 gm[2]; cornstarch, 53 gm; yeast[3], 5 gm; agar-agar, 2 gm; sodium chloride, 1 gm; Osborne and Mendel salt mixture, 4 gm; butterfat, 8 gm; and cod liver oil, 2 gm. The pork muscle from fresh defatted hams was ground, canned in tin, and autoclaved for 65 minutes at 15 pounds pressure. Before the pork was incorporated into the ration it was spread on shallow Monel metal trays and dried to $\frac{1}{2}$ its original weight at a temperature that did not exceed 180°F. Approximately one hour was required. The ingredients of the ration were mixed for 25 minutes in a Hobart mixer, after which the ration was refrigerated. A quantity of diet sufficient to meet the needs of the experimental group for three days only was prepared at any one time. Similarly, any ration remaining in the food cups after 24 hours was weighed and discarded.

Since resorptions were appearing more frequently among the animals fed the Pork I ration than had been the case in earlier studies, 1 mg of alpha-tocopherol acetate was given orally for the first 10 days of pregnancy to rule out any criticism that the animals were not receiving enough tocopherol to meet the needs of normal reproduction.

The modified Steenbock diet was composed of the following items: yellow cornmeal, 56 gm; crude casein, 5 gm; linseed meal, 16 gm; ground alfalfa, 2 gm; sodium chloride, 0.5 gm; calcium carbonate, 0.5 gm; yeast[4], 9.5 gm; yeast (irradiated), 0.5 gm; and wheat germ, 10 gm. To each 100 gm of the dry

[2] Equivalent to 15 parts protein.

[3] Northwestern Yeast Co., Chicago, Ill.

[4] Pabst IV.

ration[5] were added 33.3 gm of dried whole milk[6]. The ration was further fortified with 5 gm of ground round of beef[7] and 10 gm of fresh carrot[8], each fed three times weekly. Fifty mg of cod liver oil were fed daily in a supplement cup.

Mating of animals

The animals in groups 3 and 4 were mated when they were 10 to 11 weeks of age. Cells from the vagina were examined daily thereafter until pregnancy ensued. Positive matings were established by identification of sperm in the vagina or by the presence of a vaginal plug. The animals were sacrificed as near the termination of pregnancy as possible.

Numerous observations in earlier studies had shown that females fed the Pork I diet rarely weaned their young, while mothers receiving the Steenbock XV ration almost always were able to do so. By eliminating the first litters born to females in groups 4a and 4b, it was possible to evaluate the two rations as to adequacy for reproduction alone. These females were remated immediately for a second pregnancy.

Preparation of samples for analysis

The animals were killed by injection of sodium pentobarbital. The hair and skin were removed as rapidly as possible and these tissues, together with the intestinal tract and its contents, were weighed and discarded. The liver was excised and weighed, and the entire organ mixed in a Waring Blendor with acetate buffer (pH 4.6-4.8). The suspension was diluted with distilled water to a final weight of approximately 250 gm.

[5] The trace elements potassium iodide, manganese sulfate, potassium aluminum sulfate and copper sulfate were added to the dry basal ration.

[6] Klim. This amount was equivalent to approximately 25 ml of reconstituted milk and was the amount offered routinely to all pregnant stock animals in this laboratory.

[7] Ground round of beef purchased in advance and stored at 20° F.

[8] Fresh carrots purchased in advance and stored at 20° F.

In the case of the pregnant animals, the fetuses were removed from the uterus, weighed, and carefully examined. The total fetal tissue was blended in the Waring Blendor.

Carcass weight was determined by difference and was equal to the total weight of the animal minus the weight of the hair, skin, intestinal tract, liver, and in certain cases the fetuses. The carcass was ground in a meat grinder two or three times, after which the entire tissue was transferred to a Waring Blendor for more thorough mixing. All tissues were transferred to ½ pint Kerr jars, covered with a thin layer of

TABLE 2

Average daily intake of vitamins of rats during pregnancy

VITAMIN	VITAMIN INTAKE			
	Steenbock XV		Pork I	
	1st pregnancy	2nd pregnancy	1st pregnancy	2nd pregnancy
	μg	μg	μg	μg
Riboflavin	90	87	36	37
Niacin	787	699	528	554
Biotin	2.71	2.69	0.38	0.40
Pantothenic acid	1088	1047	131	137
Thiamine	145	140	130	136

toluene and frozen at — 10°F. The samples were stored in the frozen state until analyses could be made.

Analytical procedure

The tissues, rations, beef, and carrots were prepared for the various vitamin assays as follows: riboflavin, hydrolysis with 0.1N H_2SO_4; niacin, hydrolysis with 1N H_2SO_4; biotin, hydrolysis with 4N H_2SO_4 for two hours; pantothenic acid, enzymatic digestion at pH 4.6 with Mylase P; and thiamine, digestion with a combination of takadiastase and papain. The microbiological method was used for all assays except thiamine, which was determined by the Hennessy method ('42). The basal medium used was that of Landy and Dicken ('42).

RESULTS AND DISCUSSION

Vitamin intake

The respective quantities of each vitamin consumed by the experimental groups during the first and second pregnancies are given in table 2. The Pork I diet provided markedly less pantothenic acid and biotin, somewhat less riboflavin and niacin, and approximately the same amount of thiamine as was furnished by the stock ration. It should be mentioned that the amount of food consumed by the 10 females of a given group agreed closely, and that there was very little change in total food consumed as pregnancy progressed.

TABLE 3

Average weight of hepatic, "carcass," and fetal tissues

GROUP	LIVER	"CARCASS"	NUMBER OF YOUNG PER LITTER	FETUSES
	gm	*gm*	*gm*	*gm*
3a Steenbock XV	10.0	143.9	8.6	34.7
4a Steenbock XV	10.3	160.2	10.3	42.5
3b Pork I	9.7	146.4	4.7	17.8
4b Pork I	9.5	162.0	7.7	28.8
"Toxic" animals	7.2	150.9	11.7	3.6

General observations

The animals fed the two experimental rations grew at the same rate in the 4-week interval preceding pregnancy, the average weight gains of each group being 76 and 78 gm. Variation was observed, however, in the response of the animals to the two dietary regimens during pregnancy. The two litters produced by females receiving the Steenbock XV ration looked normal in every respect.

The reproductive performance of females fed the Pork I diet was unsatisfactory. Two of the 20 females developed the pregnancy disorder resembling toxemia, several animals resorbed their young, and the number of young borne by the females of groups 3b and 4b averaged only 4.7 and 7.7.

Observations relating to the respective weights of the maternal carcass, the maternal liver and the fetuses produced are shown in table 3. The small size of the livers of the toxic animals is characteristic (Armstrong, '39). All livers of rats fed the pork-containing diet were mottled, yellow, and somewhat friable.

TABLE 4

Average concentration of vitamins in the tissues of rats at parturition

VITAMIN	DAILY VITAMIN INTAKE	RATION	PREGNANCY	LIVER	"CARCASS"	FETUSES
	μg			μg/gm	μg/gm	μg/gm
Riboflavin	90	Steenbock XV	1	21.4	2.4	2.6
	87	Steenbock XV	2	24.3	2.3	2.5
	36	Pork I	1	18.4	2.3	1.9
	37	Pork I	2	19.7	2.3	1.8
		"Toxic" animals	–	27.0	2.1	1.5
Niacin	787	Steenbock XV	1	112.9	50.9	28.6
	699	Steenbock XV	2	109.1	54.9	30.4
	528	Pork I	1	122.5	54.6	32.4
	554	Pork I	2	118.1	49.3	28.9
		"Toxic" animals	–	151.0	48.6	14.1
Biotin	2.71	Steenbock XV	1	0.92	0.074	0.119
	2.69	Steenbock XV	2	0.92	0.058	0.101
	0.38	Pork I	1	0.67	0.068	0.057
	0.40	Pork I	2	0.70	0.055	0.053
		"Toxic" animals	–	0.87	0.065	0.032
Pantothenic acid	1088	Steenbock XV	1	89.2	6.4	37.7
	1047	Steenbock XV	2	70.2	6.0	32.1
	131	Pork I	1	45.4	6.5	37.8
	137	Pork I	2	39.7	5.8	30.9
		"Toxic" animals	–	69.4	6.9	16.3
Thiamine	145	Steenbock XV	1	10.5	1.2	2.4
	140	Steenbock XV	2	10.4	1.1	2.4
	130	Pork I	1	6.8	1.3	2.2
	136	Pork I	2	6.9	1.3	2.1
		"Toxic" animals	–	8.0	1.1	..

Only data on the occurrence of the vitamins in the maternal and fetal tissues of groups 3 and 4 will be presented at this time. These figures are given in table 4. Information on the concentration of the vitamins in tissues of younger animals will be reported in another paper.

Riboflavin

The normal reproductive performance of females receiving the Steenbock XV ration suggests that the daily intake of 90 μg of riboflavin during pregnancy provides enough and possibly some surplus of this factor for normal reproduction. The fact that the respective concentrations of riboflavin in the fetal tissues and the maternal hepatic tissues agreed so closely at the termination of both the pregnancies is additional evidence that 90 μg of riboflavin is ample for reproduction.

On the other hand, the 36 μg of riboflavin provided daily by the Pork I ration did not allow deposition of riboflavin in hepatic or fetal tissues equal to that characteristic of animals receiving the stock ration. One might conclude, then, that the lower intake was not optimum during pregnancy. The difference in riboflavin intake provided by the two rations, however, seemed to have no influence on the concentration of this factor in the "carcass" tissues. This finding is in agreement with the work of others; the concentration of vitamins in the liver is a better indication of the state of nutrition of the animal than the vitamin content of other tissues.

The 4 females which developed toxemia of pregnancy contained approximately the same total quantity of riboflavin in their livers as the other animals receiving the Pork I diet, but due to the smaller weight of their livers the concentration of vitamin per gram of fresh tissue appears high. Since the young produced by such females were representative of fetal tissue on the 16th and 18th days of pregnancy and no young of toxic animals were available on the final day of pregnancy, no information was obtained on the effect of the toxic state of the female on the concentration of this vitamin in the fetal body.

While the low intake of riboflavin characteristic of animals receiving the Pork I ration may have had some bearing on the outcome of pregnancy, and particularly on the condition

of the young at birth, the degree of deficiency of this factor appears to be minor when compared with findings relating to biotin and pantothenic acid.

Niacin

No significant differences were found between the concentrations of niacin in the hepatic, "carcass", or fetal tissues of rats receiving the two types of rations, even though these rations provided quite different quantities of the vitamin. Regardless of the niacin intake of the mothers or the average number of young produced, the concentration of this factor per gram of fresh fetal tissue was strikingly constant, 28.6 to 32.4 μg per gram. This uniformity in the niacin content of the tissues was not surprising in view of the work of Ershoff ('46) on the dispensability of dietary niacin for reproduction. The present data seem to indicate that the niacin concentration of the tissues was normal in animals fed both rations, and that the disorders of reproduction observed in rats fed the Pork I ration were not related in any way to the niacin content of the diet or to the concentration of this factor in the various tissues. It was interesting to find that the embryonic tissues of toxic mothers on the 16th and 18th days of pregnancy contained very small amounts of niacin. It is regretted that no full-term young of toxic animals were available for comparison. Possibly the low concentration of this factor in the young of toxic females on the 16th and 18th days of pregnancy is typical of normal embryonic tissues at this age.

Biotin

The wide difference in the amount of biotin provided by the two rations, and the finding that the maternal stores of the animals fed the Pork I diet were low in this factor, lead one to suspect that a biotin deficiency was an influencing factor in the disturbances of pregnancy noted among animals receiving the Pork I ration. Since the young of such females contained only 50% as much biotin as did the litters of moth-

ers fed the Steenbock XV ration, it is likely that a deficiency of this vitamin also was important in affecting the survival of the young. This finding may explain the high mortality rate observed for the young of females fed this ration.

The limited intake of biotin provided by the Pork I ration may also prove of importance through its interrelationship with pantothenic acid, which also was low in this ration.

Pantothenic acid

The Steenbock XV diet was especially rich in pantothenic acid, providing over 1 mg of the vitamin daily as calcium pantothenate. Animals consuming this ration averaged approximately 89 μg of pantothenic acid per gram of hepatic tissue at the end of the first pregnancy and their young contained over 37 μg of the vitamin per gram. This high concentration of pantothenic acid noted in the young of control animals is in agreement with the findings of Henderson et al. ('42) and Unna and Richards ('42), who have suggested a high requirement for this vitamin in the young animal. When the rats were continued on the control diet for a second pregnancy, the concentration of the vitamin in the hepatic tissue diminished from 89 to 70 μg per gram. At this time the average concentration of pantothenic acid in the fetal tissue was 32 μg per gram.

In the case of the animals receiving the Pork I diet which supplied only 130 to 140 μg of pantothenic acid per day, hepatic stores of the vitamin were markedly reduced, only 45 μg per gram being present at the end of the first pregnancy. The concentration of this factor in the young, however, was comparable to that in the young of females fed the control ration. The maternal stores at this intake of pantothenic acid seemingly were being used in an effort to maintain high concentrations of the vitamin in the developing young. When the animals were remated for a second pregnancy, the liver stores of the vitamin were reduced still further (39.7 μg per gram), although the average concentration of panto-

thenic acid in the 10 second litters was still close to the normal value of the fresh fetal tissues of the control rats.

In the group of 10 pork-fed animals that underwent second pregnancies, three females produced litters containing 10, 10, and 11 young. On the final day of pregnancy, when the animals were sacrificed, it was observed that these rats had gained only 67, 53, and 48 gm respectively, in contrast to increments ranging from 80 to 100 gm in the other 7 females. Pantothenic acid assays of the fetal tissues of the three animals revealed concentrations of 23.4, 23.9, and 23.8 μg of the vitamin per gram of fresh tissue. It is believed that these three females were sufficiently depleted in pantothenic acid to be unable to transfer ample amounts of it to their developing young, and that the fetuses were being resorbed at the time the mothers were sacrificed.

Since the concentration of pantothenic acid in fetal tissue was as high as 37 μg per gram in normal animals, it was surprising to observe that the embryonic tissues of females which became toxic on the 16th and 18th days of pregnancy contained only 16 μg per gram. The small amounts of fetal tissues present in the uteri of such animals, together with their low pantothenic acid concentration, suggest that a shortage of pantothenic acid was one of the causative factors in the resorptions that were occurring.

The importance of pantothenic acid in reproduction, together with the role of the vitamin and its interrelationship with biotin, are questions which are still unsettled. Nelson and Evans ('46) have reported that pantothenic acid is needed to prevent failure of implantation, resorptions, and birth of defective litters in rats. Emerson and Wurtz ('44) noted that a biotin deficiency was aggravated by superimposing a deficiency of pantothenic acid and, conversely, that the feeding of biotin appeared to lessen the severity of the syndrome associated with a lack of pantothenic acid. These findings have afforded some interesting speculations concerning the multiple deficiency encountered in pregnant animals consuming the Pork I ration. Perhaps here is the clue

to explain the toxic pregnancy and other abnormal symptoms noted. Wright and Welch ('44) have shown that the concentrations of pantothenic acid in the hepatic tissues of rats deficient in folic acid, biotin, and pantothenic acid did not return to normal after the administration of this vitamin unless biotin and folic acid were fed simultaneously. The findings of Pilgrim et al. ('42); Dorfman et al. ('42); Hills ('43); and Novelli and Lipman ('47) on the importance of pantothenic acid in the removal of pyruvic acid, together with those in the recent paper by Shive and Rogers ('47) relating to the function of biotin in the carboxylation of pyruvic acid to oxalacetic acid, may be the beginning of a better understanding of the role of these two factors in metabolism.

The development of the dramatic symptons of toxemia encountered in 4 animals during these experiments is difficult to understand. Histological examinations of the tissues suggest that these changes may be brought about by some toxic substance produced in the resorbing fetal tissue (Armstrong and Swanson, '43). The fact that in almost every case in which females developed the pregnancy syndrome the animals were producing large numbers of young, coupled with the observation that the disorder appeared near the termination of pregnancy, may mean that the large fetal mass depleted of vitamin could not be completely resorbed without damage to the deficient mothers. A third possibility may be that shortages of biotin and pantothenic acid induce metabolic changes in the maternal rat of sufficient magnitude to cause death. On the other hand, pregnancy may merely increase the animal's need for these two factors. Also, the shortage of pantothenic acid may have damaged the adrenal gland sufficiently to produce the symptoms of toxemia directly. Histologic studies (Molsberry, '43) of the glands have shown that acute hemorrhagic necrosis accompanies the appearance of the toxic syndrome. These various possibilities are being studied.

Thiamine

Since the daily thiamine intake was much the same for all pregnant rats, it was surprising to find that the concentrations of thiamine in the tissues varied considerably between the groups of animals fed the two kinds of rations. The average vitamin concentration in the hepatic tissue of animals fed the Pork I ration was 6.8 μg per gram at parturition, approximately ⅔ the concentration found in animals receiving the control diet. Since thiamine, biotin, and pantothenic acid are all recognized as functioning in carbohydrate metabolism, this lower concentration of thiamine in the livers of animals receiving the Pork I diet may mean that more thiamine was needed when the biotin and pantothenic acid intakes were low. Additional information regarding this point is being collected at this time. Note should be made of other experiments to be reported shortly which show that the glucose tolerance curves of pork-fed rats are abnormal. This abnormal response to glucose administration is intensified in toxic animals.[9]

SUMMARY

In an effort to determine the cause of unsatisfactory reproduction in rats fed a ration containing pork muscle and yeast as the main sources of protein and the B-vitamins, the concentrations of thiamine, riboflavin, niacin, biotin and pantothenic acid have been measured in the diets and in the tissues of pregnant animals. The occurrence of the 5 vitamins in hepatic, carcass, and fetal tissues on the final day of 1st and 2nd pregnancies has been determined in animals fed two types of rations. A modified Steenbock stock ration was used as the control diet, since it was known to promote normal reproduction.

Vitamin assays of the rations revealed that the animals which showed poor reproductive performance were consuming quantities of thiamine equal to those fed the modified stock diet, but that their riboflavin intake was somewhat

[9] Swanson et al. To be published.

lower than that of animals fed the control ration. The greatest differences in vitamin intakes were observed in respect to biotin and pantothenic acid; the inadequate diet provided only 15% as much of these factors as was supplied by the stock diet.

The concentration of vitamins in the tissues indicated that a desirable intake of riboflavin during reproduction falls in the range of 36 to 90 μg per day.

Animals fed either ration contained large amounts of niacin in their tissues, and the concentration of this factor seemed independent of the dietary intake.

The concentration of biotin in both the maternal liver and the young of animals fed the ration providing minimal supplies of biotin was subnormal.

Animals receiving the inadequate ration were markedly depleted in pantothenic acid as judged by the low concentration of this factor in the hepatic tissue. They appeared to transfer large amounts of this vitamin to the developing young until severe maternal depletion occurred.

When dietary inadequacies of both biotin and pantothenic acid occurred, as seemed to be the case when the Pork I ration was given, the animals lost the ability to store thiamine in customary amounts in their livers, after dietary supplies had been transferred to the fetuses. Perhaps in a simultaneous deficiency of biotin and pantothenic acid a larger intake of thiamine is needed to permit deposition of normal quantities in the liver.

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REPRODUCTION AND LACTATION OF RATS RECEIVING PORK DIETS[1]

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Purified rations have been found adequate for the complete life cycle of the rat, though a number of investigators have reported improvement in the lactation when various naturally occurring foodstuffs were added to the ration (Richardson and Hogan, '45; Vinson and Cerecedo, '44; Nelson and Evans, '47a; and Sporn, Ruegamer and Elvehjem, '47). Rations composed of corn, soybean oil meal, alfalfa leaf meal and minerals supplemented with the recognized nutrients have supported less satisfactory lactation than the purified rations (Spitzer and Phillips, '46). The requirements for the rat during reproduction and lactation have recently been reviewed (Schweigert, '47).

In studies conducted at Iowa State College for a number of years (Swanson, Armstrong and Nelson, '43; and Swanson and Nelson, '40) another type of diet containing natural products which would be expected to be adequate failed to support rats through the entire life cycle. Rations containing autoclaved dried pork were markedly inferior for growth, reproduction and lactation to similar rations containing beef. The females receiving pork diets showed toxemia of pregnancy with frequent deaths, fatty livers, gastric ulcers, and

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complete reproductive failure in the 2nd generation. In more recent work (Swanson, '44) synthetic vitamins have replaced 5% yeast which was used in the earlier studies as the source of B vitamins. Under this diet mothers gave birth more frequently to normal young but in most cases lactation failed. Since addition of fresh liver to these rations permitted the rats to reproduce and lactate normally, it was concluded that the pork diets were deficient in some nutrient or nutrients required for these functions.

Since 1944 work has been in progress in our laboratories in an effort to compare the value of pork with beef and other sources of protein in supporting rats during the reproductive cycle. Except for a few cases, these studies have included only parental generation rats with two litters for each female. The findings, though variable and difficult to interpret, indicate that cooked pork is sometimes inferior to casein- or beef-containing rations, particularly for lactation. Frequently the performance of rats receiving pork diets is quite comparable to that of animals receiving rations with other sources of protein. Little or no difficulty has been encountered with toxemia of pregnancy or non-viable young.

EXPERIMENTAL

Care of animals

Twenty-one-day-old, weanling, female rats obtained from Sprague-Dawley were used in all studies. In the first 4 experiments 6 rats per group were used, but in experiments V and VI each group contained 8 animals. Three or 4 rats were kept in each raised screen-bottomed cage. They received the experimental rations ad libitum, until removal to individual cages 5–7 days prior to parturition. Weights were recorded once each week until after mating and at least every three days during the gestation period.

In the first three experiments the females were mated with stock-colony males at 80 days of age. Mating was delayed until 100 days of age in the later experiments. Pregnant

females were placed in individual cages with a sheet metal floor and wood shavings for a nest. The young were counted, but not handled until the day following birth, when the mother and young were weighed and the number of young reduced to 6 per litter. Weights of young and mothers were recorded at weekly intervals, i.e., on the 8th, 15th, and 22nd days following parturition. The young were weaned on the 22nd day. The females were continued on the same ration in most cases and mated again one month later.

Composition of rations

Minor changes in the methods of compounding, handling and storing rations were made as the studies progressed. These changes are described in the paragraphs which follow. All rations were compounded isocalorically to maintain a constant protein content and the same mineral and vitamin composition, exclusive of these elements furnished by the meats.

The basal ration had the following composition: Meat or casein to provide 15, 24 or 30 gm of protein, salts IV (Phillips and Hart, '35) 4 gm, corn oil 5 gm, sucrose to make approximately 410 calories, thiamine hydrochloride 0.25 mg, riboflavin 0.30 mg, pyridoxine 0.25 mg, nicotinic acid 1.5 mg, DL-calcium pantothenate 2 mg, choline chloride 100 mg, inositol 100 mg, para-aminobenzoic acid 30 mg, biotin 0.01 mg, pteroylglutamic acid 0.02 mg, and α-tocopherol 10 mg. In addition, two drops weekly of a mixture containing corn oil, halibut liver oil, α-tocopherol and menadione were given. The daily intakes of fat soluble vitamins from this source were approximately 400 I.U. of vitamin A, 4 I.U. of vitamin D, 0.5 mg of α-tocopherol and 0.04 mg of menadione.

In the first two experiments the water soluble vitamins were added to the ration in solution at levels equivalent to those shown above, except that biotin was added at 0.002 mg per 100 gm and no pteroylglutamic acid was used. The nicotinic acid was increased from 0.25 mg to 1.5 mg per 100 gm at the beginning of experiment V. The additional α-tocopherol ad-

ministered by dropper was begun with the 2nd litter of experiment III and continued throughout subsequent experiments. The vitamin levels were varied in experiment VI; group 1 in this series received the above vitamins at twice the concentrations shown above.

Preparation of meats

Meats, procured from a local packing house soon after slaughter, were roasted or otherwise processed as indicated below. Skinned, boned hams weighing approximately 7 pounds were roasted in an electric roaster at 350°F. until the meat reached an internal temperature of 180°F. This required approximately 4 hours of cooking. After trimming off the excess fat, the pork was finely ground and stored at −5° until used. Cuts of beef round were roasted in a similar manner, ground and stored. Analyses of a number of cuts of meat thus cooked indicated an average composition of: protein 33%, fat 8%, moisture 57% for beef; and protein 27%, fat 22% and moisture 48% for pork. When fresh, dried or extracted meats were fed, protein (by Kjeldahl nitrogen) fat and moisture were determined on each such preparation until such time as reasonably accurate average figures were obtained.

In experiments I and II the meats were prepared in large quantities, ground, stored for several weeks at −5°, and incorporated into the rations as needed. In all other studies the pork was procured weekly and the beef weekly or biweekly and cooked after one to three days of cold storage. Cooked meat not fed within 7–10 days after cooking was discarded. All rations were made fresh at intervals of 4–10 days and all were stored at a temperature of −5° to +5°.

The results of experiment II suggested that changes resulting from rancidity of the fat might be contributing to the poor results with pork rations. The effect of storage time on the oxidative rancidity was checked by determining the peroxide numbers of a number of meat samples and of the meat-containing rations stored for various periods of time. The

results of these determinations (table 1) indicated that roast pork, ground and stored, developed small amounts of peroxides in 6 days. In 12 days considerable oxidative rancidity had occurred. Likewise, the ether extract of roast pork showed a marked increase in peroxide content after storage. To insure that this factor would not complicate later experiments, meats were procured and cooked frequently enough to preclude the possibility of undue oxidative rancidity.

The solvent extractions required for the preparation of rations for experiments III and VI were made at monthly intervals, using meats (raw or cooked) which had been ground

TABLE 1

Peroxide numbers for meats and meat-containing rations stored at —5°

SAMPLE	PERIOD STORED	AVERAGE PEROXIDE NUMBER
	days	*per kg of fat*
Roast pork	1	4.1
Roast pork	6	3.5
Roast pork	12	35.5
Roast pork	30	47.5
Pork fat (ether extract of roast pork)	120	53
Fresh ground ham	105	6.5
Roast pork ration	5	4.2
Roast pork ration	30	22.6
Beef and pork fat ration	30	28

and dried in a thin layer in a current of air at 50°. The dried meats were covered with Skelly-solve B (50–70°) or ethyl ether and stirred for a few hours. The solids were allowed to settle and the extract decanted off. This extraction of the residue was repeated twice and the three extracts combined and concentrated *in vacuo*. Removal of the last trace of solvent was accomplished by bubbling nitrogen through the melted fat until there was no detectable odor of the solvent present. The crude fats so prepared were stored in a stoppered brown bottle in the dark at —5° until mixed with the rations. The residues were dried at 50° for a few hours, ground to a fine powder and stored at —5° in a closed con-

tainer until used. For experiment II a continuous extractor was used for the preparation of the ethyl ether extract.

RESULTS

The data on the reproduction and lactation of rats in all experiments are presented in table 2. In the 1st experiment the rations were patterned after those of Swanson et al. ('43) in that two levels of protein were provided, 15 and 30%. All meats were cooked, ground and stored until needed, sometimes for several weeks. The pork-containing rations failed to support good reproduction and lactation. A large number of the young were born dead or died soon after birth; they were small and grew poorly, especially when the mothers received the low level of protein. Impaired lactation, particularly in the 2nd litter, was evident in the groups receiving pork rations. A large number of the females in all groups contracted upper respiratory infections and died or failed to mate the 2nd time. One female receiving the 30% pork protein ration died during parturition. Except for this one case, no manifest difficulties arose at parturition and the severe symptoms reported by Swanson and Nelson ('40) were not noted.

In the 2nd experiment, one level of protein (30%) was used. The ether extract of cooked pork was added to the beef-containing ration (group 5). Group 6 differed from group 1 only in that the hams were roasted fresh each week. As in experiment I, respiratory infection was a complicating factor, reducing the number of females to one or two in some groups for the 2nd mating. For this reason, data are not presented for the 2nd litter in these two experiments.

In all subsequent experiments the incidence of respiratory infection was lower. Although minor colds, which usually cured spontaneously, occurred following the 1st litter, it was felt that they did not complicate the results. Attempts to correlate the reproduction and lactation performance with the occurrence of these infections were not successful.

For example, in experiment V, 2nd litter, approximately 33% of the beef-fed rats had pulmonary congestion as shown by autopsy immediately after losing or weaning young, while 41% of the pork-fed rats and 12% of the casein-control animals were infected. In all experiments the incidence of lung infection was approximately 30%, but rarely was it severe enough to result in weight loss by the females.

In experiment II all rations contained 24% protein, and this level of protein was adopted for all subsequent experiments. Unused rations and cooked meat were discarded one week following cooking to insure that rancidity or spoilage would not result. All uneaten rations were removed from the feed cups daily and the cups were carefully washed twice weekly. Seldom was any evidence of spoilage detected in the uneaten portions. Changes in the rations of some groups were made immediately after the 1st litter was weaned. The 4% liver extract, which had no pronounced effect on the reproductive performance of rats receiving roast pork as the source of protein in the 1st litter (group 4), was replaced by 2 ml of fresh milk per female rat per day for the 2nd litter (group 4a). The petroleum ether residue of roast pork (group 5) was replaced by the ethyl ether residue (group 5a), and the petroleum ether extract of roast pork (group 9) was replaced by the ethyl ether extract (group 9a). The level of the "B" vitamin mixture was doubled for the 2nd litter for the groups receiving the high lard ration (groups 10 and 10a).

In all groups the performance of females was better than in the two previous experiments. Nearly all became pregnant. In the 2nd mating, vaginal smears were made daily from the time of mating until pregnancy was established. Only one resorption was recorded. Change in weight appears to be an adequate indication of pregnancies and resorptions in such studies. The control group, receiving a 24% casein ration, successfully reared a major portion of the young born, giving results quite comparable to those obtained by other workers in this laboratory using animals from the same source (Sporn et al., '47). Furthermore, most of the females receiving ra-

TABLE 2

Summary of reproduction experiments

EXP. NO.	GROUP NO.	RATION COMPOSITION	REPRODUCTION: % FEMALES HAVING YOUNG			VIABILITY: % OF YOUNG ALIVE AT 24 HOURS			LACTATION: % OF YOUNG KEPT WHICH WERE WEANED			FRACTION OF FEMALES WEANING 4–6 YOUNG [1]		WEIGHT OF YOUNG AT 1 DAY	WEIGHT OF YOUNG AT WEANING
			1st litter	2nd litter	Ave.	1st litter	2nd litter	Ave.	1st litter	2nd litter	Ave.	1st litter	2nd litter	Ave. both litters [2]	Ave. both litters [2]
														gm	gm
I	1	Roast pork, 15% protein	100	80	90	80	20	50	25	0	12	1/6	0/4	5.3	26
	2	Roast beef, 15% protein	83	43	62	70	80	75	73	100	87	3/5	1/2	5.9	34
	3	Roast pork, 15% protein + corn meal	100	100	100	90	41	65	63	55	54	2/5	1/5	5.7	38
	4	Roast pork, 30% protein	83	20	52	75	25	50	48	0	24	2/5	0/1	5.6	41
	5	Roast beef, 30% protein	100	40	70	95	80	87	88	100	94	5/6	2/2	5.7	42
	6	Casein control, 24% protein	100	100	100	97	98	98	81	83	82	5/6	4/5	6.0	37
II	1	Roast pork	60	20	40	92	100	96	71	100	86	2/3	1/1	5.3	31
	2	Roast beef	80	25	52	42	20	31	60	..	..	1/4	1/1	5.5	34
	3	Roast pork + corn meal	0	0	0	0	0	0	..	..	..	...	...	..	..
All 30% protein	4	Ether res. roast pork (dried)	100	100	100	91	97	94	79	96	87	4/5	4/5	5.7	36
	5	Roast beef + pork fat (ether ext.)	50	20	35	45	67	56	44	..	..	1/3	0/1	5.1	37
	6	Fresh roast pork	100	25	63	83	36	60	49	..	..	3/8	0	5.1	39
III	1	Frozen pork (uncooked)	85.7	80	83	63	53	58	58	100	79	2/6	2/4	5.9	48
	2	Dried pork (uncooked)	66.7	100	83	67	43	55	89	55	72	3/4	2/6	5.8	47
	3	Roast pork	66.7	100	83	97	73	85	92	68	80	4/4	3/5	5.8	45
	4	Roast pork + 4% 1:20 LE [3]	100	...	100	83	..	83	64	..	64	3/6	...	5.6	47
	4a	Roast pork + 10 ml milk/day	...	100	100	..	72	72	..	44	44	...	2/6	5.2	44
	5	Pet. ether res. roast pork	83	..	83	67	..	67	84	..	84	3/5	...	5.9	40
	5a	Ether res. roast pork	...	100	100	..	37	37	..	0	0	...	0/4	4.5	..
All 24% protein	6	Pet. ether res. dried pork (uncooked)	100	100	100	81	41	61	75	83	79	3/5	2/4	5.9	41
	7	Casein control	100	100	100	76	68	72	74	73	74	4/6	4/6	5.7	36
	8	Casein + pet. ether ext. dried pork	100	100	100	79	58	68	79	54	67	4/6	2/6	5.7	39
	9	Casein + pet. ether ext. cooked pork	100	...	100	79	..	79	34	..	34	2/6	...	5.6	41
	9a	Casein + ether ext. cooked pork	...	83	83	..	88	88	..	62	62	...	3/5	5.4	41
	10	Casein + 23% lard	67	...	67	46	..	46	8	..	8	0/4	...	5.4	18
	10a	Casein + 23% lard doubled vit.	..	100	100	..	61	61	..	67	67	...	2/5	6.3	42
All [illegible]	IV 1	24% casein, 5% corn oil	100	100	100	93	100	96	74	92	83	4/6	6/6	6.1	41
	2	24% casein, 5% corn oil + [illegible] lard	100	100	100	76	88	82	84	88	86	5/6	5/6	6.5	47
	3	Roast pork ration + vit. + 0.5 gm liver/day	100	100	100	83	90	87	75	95	85	6/8	7/8	6.1	40

	10a	Casein + 23% lard doubled vit.		100	100	..	61	61	..	67	67	...	2/5	6.3	42
IV	1	24% casein, 5% corn oil	100	100	100	93	100	96	74	92	83	4/6	6/6	6.1	41
All	2	24% casein, 5% corn oil + 23% lard	100	100	100	76	88	82	84	88	86	5/6	6/6	6.2	42
	3	Roast pork doubled B-vit. + 0.5 gm liver/day	100	100	100	83	90	87	75	95	85	6/8	7/8	6.1	40
All 24% protein	4	Roast pork	100	100	100	78	72	75	30	22	26	3/7	1/6	5.9	42
	5	Roast pork doubled B-vit.	100	100	100	68	82	75	30	30	30	2/8	1/6	5.7	40
	6	Roast pork doubled B-vit. + 0.5 gm liver	100	100	100	93	76	84	55	70	63	4/7	4/7	6.2	41
	7	Casein + 5% corn oil	100	100	100	61	90	76	15	62	38	1/8	3/7	5.6	40
	8	Casein doubled B-vit.	100	100	100	89	90	90	50	63	57	3/8	5/8	5.8	33
	9	Casein doubled B-vit. + 5 gm liver	100	100	100	86	94	90	67	67	67	5/7	5/7	5.9	33
VI	1	Roast pork doubled B-vit.	88	100	94	70	76	73	85	92	89	4/7	5/6	6.9	59
	2	Roast pork with levels of 6 B-vit. adjusted. No added 4 B-vit.[4]	75	80	78	62	76	69	83	88	86	4/6	4/4	6.3	50
	3	Roast pork with levels of 6 B-vit. adjusted and 4 B-vit. at regular levels	100	100	100	86	84	85	97	95	96	7/8	7/8	6.9	57
	4	Pet. ether residue of roast pork with adjusted B-vit. as in grp. 3	88	100	94	92	89	91	93	80	87	7/7	6/7	6.6	46
All 24% protein	5	Roast pork + 5% alcohol extracted casein [5] (vit. as in grp. 3)	88	100	94	84	72	78	100	100	100	6/7	5/8	6.9	54
	6	Roast pork + 10% fresh beef liber (vit. as in grp. 3)	88	84	86	66	95	81	100	70	85	4/7	4/6	6.8	55
	7	Roast pork + 1% liver ext.[6] (vit. as in grp. 3)	88	100	94	90	64	77	100	100	100	6/7	4/6	7.0	57
	8	Roast pork (12% protein) + roast beef (12% protein)	88	75	82	93	100	97	100	83	92	7/7	6/6	6.4	55
	9	Casein + 6 B-vit. doubled + 4 B-vit. single level	88	100	94	95	84	90	95	80	88	7/7	6/8	6.9	44

[1] Denominator of fraction is the number of females which gave birth to young.

[2] Figures for one litter only for experiments I and II.

[3] Wilson's 1:20 Liver Powder.

[4] See text.

[5] SMACO purified casein was extracted 10 times by refluxing 24 hours with 3–4 volumes of 95% ethanol each time.

[6] Sharpe and Dohme product no. 2505.

tions containing pork or preparations of pork did almost as well. From 80 to 100% of the mated females gave birth to young, and 55 to 80% of the young survived 24 hours. The lactation was fair, one-half to two-thirds of the females weaning 4–6 young. Exceptions were the animals receiving the ethyl ether residue of cooked pork, the petroleum ether extract of the cooked meat, and those receiving the milk supplement. In these cases, one-third or less of the females weaned 4–6 young. Doubling of the vitamin supplement added to the high lard ration caused marked improvement, especially in lactation. Whether the requirements of some vitamins for lactation are higher than the amounts fed when the ration contained much fat, or there was enhanced destruction of one or more vitamins during the one-week storage of them already mixed in the ration, has not been determined. That one of the two was involved is indicated by the differences in the results obtained with groups 7 and 10.

In the next experiment (IV), started while experiment III was still in progress, high lard diets were fed in an effort to determine if the high fat level was resulting in oxidative changes. Five per cent corn oil rations were compared with high levels of stabilized and non-stabilized lard.[2] The lard replaced approximately 60% of the sucrose isocalorically to give a ration containing 30% fat by weight (23% lard). The B-vitamins were doubled in all groups. In this experiment, and in the two which followed, the 1st mating was delayed until the females reached 100 days of age. All of the females became pregnant, most of them produced normal, healthy young, and most of the young survived for 24 hours. The lactation index was only 42% for the 2nd litter in the group receiving stabilized lard. The reason for this inferiority was not evident. Three of the females lost all of their young in the 1st week and none had a respiratory infection. The performance of the casein-corn oil control group was better than in the previous experiment, possibly because of the more

[2] Kindly supplied by Armour and Company, Chicago, Illinois.

advanced age at the time of mating.[3] The results indicated that when fat is substituted isocalorically for carbyhydrate it does not markedly affect the reproductive functions of rats receiving 24% casein rations in the presence of adequate levels of vitamins.

Because experiment III (groups 10 and 10a) indicated increased destruction of, or increased requirement for one or more B-vitamins in rations high in lard, the next experiment was planned to ascertain the effect of supplementing casein, beef and pork rations with addition of B-vitamins and fresh liver (see table 2). It will be noted that in contrast to the results of experiment III, animals receiving pork rations failed to lactate properly in both litters. Doubling the B-vitamins had no effect, while 0.5 gm of fresh beef liver per day resulted in some improvement in this function. Inferior lactation in the 1st litter by females receiving the casein control ration was improved by doubling the vitamins, and was further improved by supplementing with fresh liver. Increased vitamin levels appeared to increase the mortality of young and to decrease the lactation of the females receiving the roast beef.

The observation that increasing B-vitamin levels had a detrimental effect on the beef-fed rats but improved the casein and pork-fed rats, and the findings of Richards ('45), indicate the possibility of an imbalance of vitamins being responsible for the inferiority of pork to beef and casein in certain experiments. This possibility was investigated in experiment VI.

In the previous experiments all of the vitamins were added without correcting for those provided by the meats in the rations. In this experiment, B-vitamins were added to the rations, as indicated in table 2, in three different patterns: (1) At twice the regular levels in addition to those provided by the meats; (2) only thiamine, riboflavin, nicotinic acid,

[3] Rats from this source receiving stock diets have been found to mate and reproduce more successfully if they are at least 100 days of age (R. K. Meyer, unpublished).

pyridoxine, calcium pantothenate and choline at twice the regular levels, taking into account the amounts of these 6 vitamins present in the meats; and (3) the same as (2) except that regular levels of biotin, folic acid, inositol and *p*-aminobenzoic acid were provided in addition to those furnished by the meats. Other variations included the replacement of a portion of the pork by casein and beef, and supplementation of the pork rations with fresh liver or a liver extract. In contrast to experiment V, lactation was good in all groups (85% or more). In some groups one or two females failed to become pregnant and in a few instances the young were less viable. Unusually good performances in all respects were obtained with casein, a pork-beef mixture or petroleum ether-extracted pork as the protein sources. The improved performance was also reflected in larger young at birth and at weaning than had been obtained previously (table 2). The young from mothers on lower fat rations (groups 4 and 9) were smaller than those in other groups. There is some indication that the young survived the first two days of life better when the mothers were smaller, but this correlation does not exist in all groups. The uniformly good results in this experiment prevented the observation of any possible influences of the vitamin imbalance which may exist in these rations.

From the more or less uniform results obtained with casein and beef in the last 4 experiments, and the extremely variable results with pork, it seems probable that the quality of the pork was an important factor in these studies. In experiment III the roast pork appeared to maintain nearly an adequate nutritional state for the reproductive cycle; in experiment V, however, roast pork failed to support good lactation unless supplemented with fresh beef liver. Again, in experiment VI roast pork supplied the necessary nutrients for a relatively good reproductive performance. It should be pointed out that group 1, experiment VI, and group 5, experiment V, received identical rations except that different batches of meat were used. The cuts of meat used in all experiments except I and II were obtained from market hogs and were purchased often

enough to represent a cross section of the hogs sold in this locality at the time.

The growth rates of the females receiving pork rations were never inferior to groups in the same experiment receiving other rations. Often they grew slightly better than the control groups receiving casein as the protein. The average weekly growth rate was approximately 25 gm (20–28 gm) for all experiments during the first 6 weeks. The average number of young born per female was also not significantly affected by the rations in these studies. The averages in most groups were 7–9 young per litter, the 2nd litter containing the same number or slightly fewer than the 1st.

DISCUSSION

Why pork should be inferior to beef in the nutrition of the rat is not evident. The most notable difference in gross composition, the lipid content, does not appear to be the explanation, since lard at a fat level comparable to that provided by pork was not detrimental when casein was the protein. Confirming the findings of Swanson and Nelson ('40), rancidity of fat present in the pork rations is not responsible for this inferiority. Deuel et al. ('47) report better reproduction and lactation with some fat in the ration. Increasing the level above 5% caused no improvement, but resulted in slightly higher mortality during the nursing period. Anthony ('44) reported satisfactory reproduction and lactation on a low fat diet. When rice bran concentrate was used as the source of water soluble vitamins and 1% linoleic acid as the sole lipid source, satisfactory growth, reproduction and lactation were obtained. When rice bran concentrate was replaced by the crystalline vitamins, satisfactory reproduction was found with 1–2% ethyl linolate, but growth and lactation were improved by addition of 30% butterfat or cottonseed oil. Nelson and Evans ('47b) reported a deleterious effect on lactation when the fat content of the ration was increased. They did not make their substitutions of fat for carbohydrate on an isocaloric basis, however, and the influence of increased fat

levels on lactation may have been the result of the effective lowering of protein, salts or vitamins. These ingredients were reduced to approximately ⅔ of the level fed on the high carbohydrate and high protein diets when calculated on an isocaloric basis.

The protein quantity and quality do not appear to be responsible for the difference between pork and beef. Swanson and Nelson ('40) found no significant change in the biological value of pork protein during cooking. Addition of liver, which did not alter the biological value, improved the nutritive value markedly as judged by reproduction and lactation performances. Pork and beef protein appear to be very similar in amino acid composition and in biological value (Block and Mitchell, '47).

The variation from one experiment to another and the improvement in lactation when liver was added to the pork rations indicate a dietary inadequacy or imbalance. The improvement of lactation on casein rations by doubling the B-vitamins (groups 7 and 8, experiment V) and failure to get improvement by this means with the pork rations suggest again an imbalance of vitamins or the presence of a substance or substances in casein and beef not present in pork muscle.

Attempts to use the short-term method (Nelson and Evans, '47b) of determining the effects of supplements on lactation were not successful in these studies. Weight increases of the mothers shifted from stock diets to the meat-containing rations at parturition were considerable in all cases. The variable results with pork may be related to the fact that hogs are monogastric animals and the pork muscle may not be supplied with ample quantities of unknown factors produced by the microorganisms in the rumen of ruminants.

It should be pointed out that these results cannot be applied to human nutrition, since no one would receive all of his protein from pork, nor would he consume pork at every meal. The experiments reported here are abnormally balanced in order to produce conditions which will allow demonstration of the differences between pork and other materials as sources

of protein. The variable factor may be the same as that encountered in several other studies, with various types of non-purified rations.

SUMMARY

Female rats fed rations containing pork as the source of protein mated normally and gave birth to apparently normal young, but in some cases failed to rear them. The primary defect appeared to be in lactation. Whereas casein-fed rats usually reared an average of approximately ⅔ of the young and beef-fed rats 80–90%, pork-fed animals reared less than ⅓ of their young in certain experiments, indicating poor lactation. In other experiments the lactation was comparable to that obtained with rations containing beef.

When lactation failures were encountered with pork rations, increasing the B-vitamins had no effect, but fresh liver restored the lactation to that obtained when beef or casein was fed.

The levels of B-vitamins commonly used in growth experiments were found inadequate for optimum reproduction and lactation performance with casein as the source of protein, especially when the fat level was high.

Attempts to obtain a defatted pork preparation which would approach the beef or casein rations in supporting lactation were not successful.

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THE CHOLESTROL CONTENT OF COWS' MILK[1]

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During the past few years interest in cholesterol as it relates to human physiology has markedly increased. One of the primary reasons for this is the suggested association between the development of blood vascular disorders and the cholesterol intake. A number of references in the popular press have indicated a relation between the intake of cholesterol and some diseases of the circulatory system (Leary, '44; Pasquarelli, '46; Dock, '47). Although many papers on this subject have appeared since Bischoff ('32) made a thorough evaluation of the available literature, his conclusion that there is very little valid evidence of the existence of any such relation still appears tenable.

In addition, cholesterol is being prominently mentioned as a precursor of various steroid hormones (Bloch, '45; Perlman and Leonard, '47), while the ester form has been suggested as the blood precursor of the fat in milk (Saarinen, '44). A great deal of work is being done on the relation of the cholesterol intake to the development of fatty livers (Loizides, '38; Best and Ridout, '36).

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The increasing interest being shown in cholesterol prompted a critical evaluation of the amount of this substance in milk. Although there are a number of reports on the level of cholesterol in cows' milk, there is no indication as to its variation in the milk from different cows or in that from the same cow on different occasions. The work reported here was undertaken to supply this information.

ANALYTICAL METHODS

The Schoenheimer and Sperry ('34) method for the analysis of free and ester cholesterol in blood was adapted for this work. In later reports, the Columbia group (Sperry, '38; Sperry and Brand, '43) suggested that the acetic anhydride and sulfuric acid be mixed and then kept cold in an ice bath until used. After a number of trials, however, it was decided to follow the older method. According to this procedure, the sulfuric acid was slowly added to the colorimeter tube containing the acetic anhydride and the digitonin precipitate which was dissolved in acetic acid. The tube was vigorously shaken while the sulfuric acid was added and the shaking continued for about a minute before the tube was placed in a water bath maintained at 25°. All readings were made with an Evelyn photoelectric colorimeter at intervals between the 27th and 37th minute after the addition of the sulfuric acid to the tube. In each case, the lowest galvanometer reading secured during this interval was used; it corresponded to the maximum color development. The cholesterol analyses were not started until experiments indicated that from 90 to 105% of the cholesterol added to milk could be recovered. All analyses were made in duplicate.

The butterfat in the milk was determined by the routine Babcock test. These analyses were made on the Farm Campus. The statistical evaluation of the data involved the analysis of variance as described by Snedecor ('46) [2].

[2] We are grateful to Dr. W. O. Caster for aid in the statistical analysis.

EXPERIMENTAL ANIMALS

The cows used in this study were a part of the regular University Farm herd. Most of them had freshened within the three-month period preceding the first milk sample, while two of them were up to a month further along in their lactation period (table 1). In the early spring, when this study was initiated, the cows were kept in the barn. The milk samples obtained at that time are labeled "winter milk." During the summer, the animals were kept on pasture supplemented with concentrates. The samples obtained

TABLE 1

Description of cows. Free and total cholesterol and butterfat in milk (The milk samples were collected on March 24)

COW	AGE	LAST CALVING	CHOLESTEROL		FAT
			Free	Total	
	years	*date*	*mg/100 ml*		*%*
Holstein					
474	9	2/20/47	9.8	9.9	2.4
480	9	2/ 3/47	9.5	9.5	3.3
838	3	1/15/47	..	..	..
Jersey					
297	4	1/ 6/47	14.1	14.1	4.9
275	6	12/26/46	15.0	15.0	5.0
293	4	12/10/46	11.5	11.6	3.9
Guernsey					
639	6	1/28/47	10.5	10.4	3.0
672	2	2/26/47	10.4	10.3	3.7
658	4	11/19/46	...	...	..

at that time are called "summer milk." Six separate samples were analyzed at various intervals in each of these periods.

While the cows were kept indoors, they received alfalfa hay ad libitum, corn silage, and a grain mixture composed of corn, oats, barley, linseed oil meal, bone meal and salts. The cows were put out on pasture May 16, 1947, at which time the winter ration was reduced by one-half.

The cows were milked twice each day. On each occasion, the milk used in this work was obtained in the morning and was a true composite of that entire milking.

TABLE 2

Cholesterol and butterfat in cow's milk [1]

Cow no.	HOLSTEIN						JERSEY						GUERNSEY					
	474		480		838		297		275		293		639		672		658 [2]	
	Ch.	Fat	Ch.	Fat	Ch.	Fat	Ch.	Fat	Ch.	Fat	Ch.	Fat	Ch.	Fat	Ch.	Fat	Ch.	Fat
Winter milk																		
3–24	9.8	2.4	9.5	3.3	7.4	2.4	14.1	4.9	15.0	5.0	11.5	3.9	10.5	3.0	10.4	3.7	12.7	4.7
4–17	9.6	2.5	9.3	2.9	7.8	3.2	12.1	3.8	14.2	5.0	9.9	1.9 [3]	12.9	3.6	9.1	3.0	12.2	3.0
4–30	8.5	2.0 [3]	8.4	2.0 [3]	7.4	1.6 [3]	12.8	4.7	12.4	4.3	16.8	5.8	13.8	4.4	9.3	1.8 [3]	12.8	3.8
5–12	9.0	2.8	8.8	2.8	8.2	3.5	13.0	4.8	15.2	5.5	11.6	1.8 [3]	14.7	4.2	9.5	3.8	12.2	4.4
5–21	8.2	3.1	11.1	4.7	7.0	3.3	11.5	5.2	13.3	5.6	15.6	4.9	16.0	4.8	12.8	4.8	11.8	4.8
5–30	9.0	2.5	9.5	2.4	8.7	2.1 [3]	14.3	4.2	15.0	5.5	13.1	4.4	11.8	3.4	12.0	3.3	12.7	5.6
Summer milk																		
7–9	10.1	2.7	8.8	2.5	8.4	3.3	12.6	4.8	11.2	5.7	13.5	4.7	8.0	4.5	9.1	3.6		
7–16	9.8	2.4	10.1	2.3	6.9	3.1	10.6	4.5	13.2	5.7	13.8	5.6	8.7	4.2	9.2	3.4		
7–23	8.3	2.6	11.7	3.0	6.7	3.2	9.3	4.7	13.2	5.2	11.6	5.5	10.3	4.4	11.7	4.0		
8–5	9.7	2.9	15.3	3.2	8.6	2.8	12.4	4.6	13.3	5.2	14.4	4.9	13.9	4.5	11.0	3.7		
8–12	16.8	3.8	16.6	3.4	8.5	3.1	11.6	6.2	13.3	4.6	12.4	4.4	10.1	4.2	11.3	4.6		
8–18	8.8	2.2	14.2	4.0	9.0	3.0	11.5	3.4	12.4	3.6	14.0	5.0	13.6	4.5	11.8	3.8		

[1] The fat is expressed as grams per 100 ml of milk. The cholesterol is expressed as milligrams of total cholesterol per 100 ml of milk.

[2] Cow 658 was sold before the experiment was completed.

[3] The values followed by [3] are explained in the text.

RESULTS

The results of the analyses of the milk for both the free and the total cholesterol are given in table 1. The agreement between the two sets of values was so good that there was no reason to believe that any ester cholesterol was present in the milk. For this reason, the subsequent analyses for cholesterol involved only the precipitation with digitonin without any preliminary saponification.

The values for the cholesterol and butterfat in the milk samples are given in table 2. The results of the statistical

TABLE 3 [1]

Variations in the cholesterol content of milk

MILK	ALL COWS	HOLSTEIN	JERSEY	GUERNSEY [2]
		Within-cow variation		
Winter	11.4 ± 1.50	8.7 ± 0.80	13.4 ± 1.98	11.9 ± 1.62
				12.4 ± 1.50
Summer	11.3 ± 1.56	10.5 ± 2.05	12.5 ± 1.01	10.7 ± 1.28
		Inter-cow variation		
Winter		8.7 ± 1.14	13.4 ± 1.93	11.9 ± 2.45
				12.4 ± 1.97
Summer		10.5 ± 3.03	12.5 ± 1.39	10.7 ± 1.17

[1] The mean values together with their deviations expressed as mg/100 ml of milk are given in each case.

[2] The first of the 2 values after Guernsey is the mean of 2 cows; the second is the mean of 3.

analysis of these data are presented in tables 3 and 4. From these it can be seen that the mean cholesterol level in the milk was 11.4 mg per 100 ml for the winter samples and 11.3 mg for the summer milk. When the values for all the cows are thus considered, there is no significant difference between the summer and the winter milk. As will be brought out later, there are some slight breed differences for the two seasons.

There is a slight day-to-day variation (variation between days, table 4) in the cholesterol content of the milk during

the summer; this variation is still less in the winter. The differences in the cholesterol level in the milk from the individual cows within the same breed are significant. This is particularly true for the summer, when the F test shows a significance beyond the 1% level.

The milk from the different breeds showed a significant difference ($P < 0.01$) in levels of cholesterol during the winter. In contrast to this, the summer milk shows no significant breed differences. This is due to the fact that the day-to-day variation was barely significant in the summer, while the differences in the cholesterol levels for the milk from cows within the same breed were so great that the

TABLE 4

Statistical evaluation of results[1]

SOURCE OF VARIATION	WINTER		SUMMER	
	F test	Significance	F test	Significance
Variation between days	0.59	No significance	2.58	*
Variation between cows	2.84	*	6.62	**
Variation between breeds	16.23	**	1.26	No significance

[1] The significance of the F test is given according to the following notation: * = between the 5 and 1% level of significance, ** = beyond the 1% level of significance.

breed differences were completely overshadowed. This is perhaps more understandable when it is realized that the mean cholesterol levels for the winter milk from the various breeds ranged from 8.7 to 13.4 mg per 100 ml, whereas in summer the means ranged only from 10.5 to 12.5 mg (table 3). In the latter period, the deviation for any mean was usually greater than the difference between the respective means. For this reason, the breed differences in the summer were insignificant.

The seasonal variation in the cholesterol content of milk was not the same for the different breeds. For the Holstein milk, the difference in the cholesterol level for the two seasons was barely significant as shown by an F test of 25

(table 4). The difference for the Jersey milk was also only slightly significant. A similar analysis for the Guernsey milk was not made since one of these cows was sold in the middle of the experiment. The biological significance of these differences is open to question, since the milk from the Holsteins showed a lower mean value in winter than in summer and the Jersey milk exhibited just the reverse. These differences for the two breeds were of the same order of magnitude, since the mean cholesterol levels for all cows regardless of breed were the same for both seasons.

There was a considerable degree of correlation between the butterfat and the cholesterol contents. The correlation coefficient for this relationship in the milk from all cows throughout the entire experiment was 0.64, which was highly significant far beyond the 1% level. For the winter milk, the correlation coefficient was 0.76, which was also highly significant far beyond the 1% level. The correlation coefficient for the summer milk was 0.45, which was significant at the 1% level. These values were obtained when the figures followed by a question mark in table 2 were included in the calculations. The butterfat values below 2% are questioned, since the accuracy of the Babcock method at such levels is not as great as at the higher levels. For this reason these values were omitted in a recalculation, but this did not alter the correlation coefficients.

DISCUSSION

The reports in the literature have indicated considerable disagreement as to the presence of any esterified cholesterol in milk. The values for the esterified cholesterol range all the way from 100% (Wacker and Beck, '21) through progressively decreasing amounts (Denis and Minot, '18; Shope and Gowen, '28; Fox and Gardner, '24) to zero, in which case nothing but free cholesterol was present in the milk (Ansbacher and Supplee, '34; Dam, '34b; Mühlbock, '34). The latter work agrees with our results (table 2). As Ansbacher and Supplee ('34) have suggested, the earlier results in-

dicating the presence of ester cholesterol in milk are probably attributable to the analytical methods used.

All of the reports are in agreement that the total cholesterol in cows' milk ranges from about 9 to a maximum of 17 mg per 100 ml (table 5). This agreement occurs in spite of the fact that some of these workers maintained that there were large amounts of ester cholesterol in milk. For instance, Wacker and Beck ('21), who claimed that all of the cholesterol in milk was present in the ester form, found

TABLE 5

Cholesterol content of cows' milk as reported in the literature

AUTHORS	METHODS USED	FAT	CHOLESTEROL
		%	*mg/100 ml*
Denis and Minot ('18)	Colorimetric (Bloor)	3.2–5.0	10–17.6
Wacker and Beck ('21)	Digitonin precipitation (Windaus)	3.65	12.58
Nakanishi[1] ('31)	—	3.85	12.67
Coccheri[1] ('32)	—		12–15
Dam ('34)	Digitonin precipitation	3.65	13.36
		3.45	12.50
Mühlbock ('34)	Digitonin precipitation	1.80	9.18
Torrisi ('40)	Liebermann. Burchard Schoenheimer and Sperry ('34)		10–12
Nataf-Mickelsen-Keys-Peterson ('48)	Holstein	2.87	9.6
	Jersey	4.70	12.9
	Guernsey	3.98	11.5

[1] It was impossible to determine the methods used by Nakanishi and Coccheri.

a value of 12.6 mg total cholesterol, which is within the range reported by others. Since the ester cholesterol is usually determined as the difference between the free and the total, it would appear as though the earlier methods for measuring free cholesterol gave values that were too low.

There is some disagreement among the various workers in this field over the question of the relation between the percentage of fat and the cholesterol content of cows' milk. Denis and Minot ('18), who were the first to consider this question, claimed that the cholesterol showed a "direct and

proportional variation'' with the butterfat. This was based on a visual inspection of their data. The product-moment correlation coefficient for their values, however, is 0.19, which is insignificant. So in spite of their contentions to the contrary, their data show no relation between the butterfat and the cholesterol levels of milk. Fox and Gardner ('24), from a study of a small number of samples from a few cows, indicated that in certain cows ''the cholesterol appears to follow approximately the output of fat.'' When they considered the over-all results of their small series, they decided there was no ''exact ratio'' between the butterfat and cholesterol content of milk. They quoted the results of Wacker and Beck ('21) to confirm their findings. More recently, Ansbacher and Supplee ('34) have claimed that ''the cholesterol content of whole milk is quite variable and not dependent upon the fat content,'' but they gave exact data for only one sample of whole milk.

The results of the previous work are at variance with our own, in which a high correlation between the butterfat and the cholesterol content was found. It is possible that the earlier workers used too few samples to see the true relation between these two variables. For instance, the largest number of such samples used in earlier analyses was in the study of Denis and Minot ('18), in which 15 separate values for cholesterol and butterfat were reported. Another factor that may aid in explaining our finding of a high degree of dependence between these two values is the spread in the butterfat levels in the milk from the present cows. The lowest butterfat value in this series was slightly below 2%, while the highest was 6.2. This is twice the range found in Denis and Minot's study ('18).

None of the previous series has been extensive enough to permit an evaluation of the influence of season upon the cholesterol content of milk. Although we found a slight difference between the levels of cholesterol in summer and winter milk, this was not the same for the Holstein and Jersey cows. If this seasonal variation is of biological signif-

icance, it is doubtful whether it can be explained on the basis of dietary changes. The only significant difference in the rations of the two seasons was the addition of fresh pasture grass to a reduced winter ration during the summer. None of the materials fed to the cows in this experiment contained any cholesterol, since only plant products were used. Cholesterol is found in animal products (Okey, '45); plants contain other related sterols and at present it is questionable whether these are absorbed by mammals (Schoenheimer, '32). Dam ('34a) has shown that even when cholesterol in fairly large amounts was fed to a goat for three days, there

TABLE 6

Relation between the cholesterol content of blood and the cholesterol content of milk. (The blood and milk samples were secured May 21st)

COW		SERUM CHOLESTEROL		MILK CHOLESTEROL
		Free	Total	Total
		mg/100 ml		*mg/100 ml*
Holstein	838	24.1	183.1	7.0
Holstein	474	12.7	91.9	8.2
Holstein	480	19.4	152.5	11.1
Jersey	297	24.1	140.6	11.5
Guernsey	658	17.8	136.2	11.8
Guernsey	672	29.1	151.5	12.8
Jersey	275	19.8	134.4	13.3
Jersey	293	19.8	121.3	15.6
Guernsey	639	20.2	86.9	16.0

was no augmentation in the cholesterol content of the animal's milk.

Until now, there has been no systematic study of the cholesterol content of milk from different breeds. The present work indicates a significant breed difference in winter which is not apparent in summer. The explanation for this variation has been considered above.

The day-to-day variation in the butterfat content of milk has been studied by the earlier workers in this field. Some of the results (Eckles, '12, '20) were more extensive than the present ones and indicated a fairly high daily varia-

tion in this factor. There are no such previous studies on the variation in the cholesterol content of the milk. Our findings indicate that the day-to-day variation of the milk cholesterol is greater in summer than in winter. It is impossible to suggest any explanation for this difference.

In the early part of this study an attempt was made to see whether there was any relation between the cholesterol level in the blood and that in the milk from the same cows. The results of analyses of the milk and the jugular vein blood secured from the same cows on the same day are given in table 6. From these data it can be seen that there is no relation between the cholesterol content of the milk and of the serum. If Saarinen's ('44) work on the serum cholesterol as a precursor of butterfat is substantiated, it must involve a more complicated series of reactions than simple hydrolysis of the cholesterol esters and the transfer of either one or both of these components through the alveoli epithelium. The present results also show that the total cholesterol in the serum is not related to breed, and that the free cholesterol ranges from 12.7 to 23.2% of the total which is approximately the same, or at most only a slightly lower percentage than that in human beings (Sperry, '36).

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THE BASAL METABOLISM OF TWO HUNDRED AND EIGHTEEN GIRLS AND YOUNG WOMEN OF SOUTHERN ARIZONA, FOURTEEN TO TWENTY-THREE YEARS OF AGE, INCLUSIVE

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ONE FIGURE

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Basal energy metabolism of native white people living in this country has been shown by a number of investigators (Tilt and Walters, '35, in Florida; Eaton, '39, in New Orleans; and others) to be lower in subtropical climates than in temperate climates.

The state of Arizona, where the present study was made, has wide variations in its geography and climate. Fully two-thirds of the northern half consists of plains at high altitudes and mountainous areas. Much of this area is more than 4000 feet above sea level where the winters are cold and summers cool. Southern Arizona, on the contrary, presents a sharply different picture, especially in the south central and southwest. This area, covered by the valleys of the Salt, Gila, and Colorado rivers, is located in the area of the United States which has the most sunshine and the least relative humidity (Yearbook of Agriculture, '41).

Few studies of basal metabolism on girls who live in subtropical climates have been reported in the literature and there are none from the state of Arizona. Selection of subjects, therefore, was confined to girls living in southern Ari-

zona, where the work was being done. The age range selected includes the years from 14 to 23, inclusive.

EXPERIMENTAL

Subjects

Basal metabolisms have been determined on 218 subjects on the basis of 887 individual tests which were made at intervals during the years 1940 to 1947 in the winter months between October and April. All subjects were white and, with few exceptions, were born and reared in southern Arizona or arrived there in early infancy. The exceptions had lived in other states with similar climates for 5 years or less, several years previously. They were taken at random from volunteers in the junior and senior high schools and the State University of Arizona.

Before being accepted as subjects they were questioned as to the possibility of their having had thyroid therapy, since we have observed that thyroid extract is administered frequently in this part of the country. Tests were made during the menstrual cycle, provided the subject was rested and relaxed.

Procedure

Using the indirect method of calorimetry, two 8- to 10-minute tests were made on each of two or more mornings, following each other as closely as possible. No preliminary tests were made. Subjects returned for further testing until oxygen consumption in cubic centimeters per minute from either of the tests on a given day agreed within 5% with the consumption on another day. Surface area was calculated from the height-weight formula of DuBois as cited by Carpenter ('39). Calories per m^2 per hour were calculated by each of 4 methods commonly used in evaluation of basal metabolism. These are: (1) average of results of all tests within 5% of 1 accepted as a base line on at least two mornings, (2) average of all accepted tests, (3) accepted first tests only, (4) average of accepted tests on the first morning only.

RESULTS

Physical data

Physical data expressed as yearly means are shown in table 1. Some cases deviated in weight more than minus 10 and plus 15% from the Wood standards as cited by MacLeod and Taylor ('44). Several of these cases, when measured by the Pryor standards ('40) which take into consideration skeletal structure, fell within a normal range. Only a few appeared to be overweight, although several were quite slender. The range of oral temperatures was 96.3° to 98.6°F. There was a wide range of recumbent pulses per minute: 44 to 85.

TABLE 1

Physical data of subjects

CASES	AGE YEARLY MEAN	WEIGHT	HEIGHT	SURFACE AREA	PULSE PER MIN. AVE.	TEMP.	VITAL CAPACITY DEV.	CALORIES PER M^2 PER HR.
		kg	*cm*	m^2		°*F.*	%	
23	14.48	52.6	162.6	1.55	65	97.9	7.9	36.22
23	15.45	54.2	165.4	1.59	66	98.3	9.0	34.37
24	16.53	55.3	165.9	1.62	64	98.1	8.3	33.12
25	17.51	56.6	166.4	1.63	66	97.9	5.5	32.12
36	18.53	55.6	164.6	1.60	62	97.8	9.4	31.21
21	19.33	56.4	166.8	1.62	64	97.7	10.2	31.12
24	20.52	57.2	167.1	1.64	62	97.8	9.5	31.14
21	21.39	60.3	166.5	1.67	62	97.7	11.2	31.30
12	22.31	57.0	167.6	1.64	62	97.3	9.2	30.84
9	23.48	60.9	166.1	1.68	59	97.7	11.5	30.79

The respective coefficients of variation of vital capacity for each year are large: 12.2, 12.7, 11.5, 12.3, 13.9, 13.0, 13.4, 16.7, 17.8, and 18.7%. Vital capacity was referred to 3 commonly used standards: (1) Dreyer ('19), based on weight; (2) West ('20) referred to surface area, (3) West ('20) based on height. Although differences are small in some years, for each year except the 23rd West's standard referred to surface area, without regard to plus or minus sign, predicted the actual vital capacity more closely than his standard referred to height. This standard also predicted more closely

than Dreyer's standard referred to weight for each year except the 15th and 20th. Mean deviations from West's standard based on surface area, without regard to algebraic sign, are therefore included in table 1.

Statistical treatment of data

Calories in table 1 are based upon the first method of evaluating data. By this method only 14% (31 cases) of the total returned on a third morning, and 2% (4 cases) on a 4th. In only 10% (21 cases) were both tests on first mornings omitted. But 50% (109 cases) of first tests on the first morning could not be included because 80 were more than 5% above any other test, 9 below, and the spirograms of the other 20 too irregular to use.

Smoothed means of the first 5 years for each of the 4 methods of evaluating data from individual tests were obtained by reduction of data to linear form (Yule and Kendall, '45). Logarithms of calories minus a constant for each year were plotted, and straight lines fitted by means of the equation log (y-c) = log a-x (log b). Y represents calories per square meter per hour; c, a constant; x, age in years; log a, the (y-c) intercept at 0 years; and log b the numerical value of the slope or yearly drop in calories. The constant used in the first method was 29.5, and in each of the other methods. 30.

Table 2 shows the smoothed means by each of the 4 methods for the years 14 to 18, inclusive, and true means for the years 19 to 23. When the data of the first 5 years were reduced to linear form, the best straight line fit was obtained by the second method; the next by the first. As was to be expected, the lowest yearly means were obtained by the first method. A sharp drop in calories was shown by all methods, the means in the first changing from 36.40 to 31.21 during the 5 years.

The coefficients of correlation with age of the weighted smoothed means are highly significant by each of the first two methods: i.e., -0.995 ± 0.0011 and -0.997 ± 0.0010, respec-

TABLE 2

Means in calories per m² per hour and variability by 4 methods of evaluating data

AGE, YRS.	CASES	CAL.	σ [1]	C.V.[2]	CASES	CAL.	σ [1]	C.V.[2]
		Five % check				*All tests averaged*		
14.00–14.99 14.48(M)	23	36.40	3.32	9.12	23	36.78	3.34	9.07
15.00–15.99 15.45	23	34.37	2.64	7.67	23	34.71	2.45	7.04
16.00–16.99 16.53	24	32.94	2.15	6.52	24	33.27	1.95	5.85
17.00–17.99 17.51	25	31.92	3.24	10.14	25	32.27	2.50	7.76
18.00–18.99 18.53	36	31.21	2.08	6.68	36	31.58	2.06	6.51
19.00–19.99 19.33(M)	21	31.12	2.45	7.87	21	31.40	2.56	8.15
20.00–20.99 20.52	24	31.14	2.99	9.60	24	31.67	2.95	9.31
21.00–21.99 21.39	21	31.30	2.26	7.22	21	31.67	2.38	7.52
22.00–22.99 22.31	12	30.84	2.37	7.68	12	31.29	2.60	8.31
23.00–23.99 23.48	9	30.79	1.32	4.29	9	31.62	1.18	3.73
		1st test				*1st morning*		
14.00–14.99 14.48(M)	22	38.40	4.03	10.48	22	36.56	2.86	7.81
15.00–15.99 15.45	21	36.16	3.06	8.45	22	34.70	2.80	8.07
16.00–16.99 16.53	24	34.52	2.55	7.39	24	33.37	2.35	7.05
17.00–17.99 17.51	21	33.31	3.78	11.36	24	32.41	3.15	9.72
18.00–18.99 18.53	34	32.43	2.38	7.34	36	31.73	2.02	6.36
19.00–19.99 19.33M	17	31.00	2.31	7.45	21	31.51	2.35	7.46
20.00–20.99 20.52	18	32.58	3.23	9.91	24	32.45	3.23	9.95
21.00–21.99 21.39	17	32.56	2.74	8.42	21	31.78	2.32	7.30
22.00–22.99 22.31	12	31.79	3.31	10.41	12	31.52	2.91	9.23
23.00–23.99 23.48	8	31.30	3.44	10.99	9	32.33	1.82	5.63

[1] Standard deviation.

[2] Coefficient of variation.

tively. By the third and 4th methods they are just barely significant, i.e., -0.929 ± 0.0080 and -0.953 ± 0.0542, respectively.

No real differences are found in the yearly means from 19 to 23 by either of the first two methods. However, by the third and 4th methods approximately half of the differences show a better than even chance that they are real. When the averages of these yearly means by each of the first two methods are compared with the means of the 18th year in each case, no real differences are found. Lumped means of 31.1 and 31.5 calories, respectively, have been taken, therefore, for the 18th to the 23rd year for each of the two methods.

DISCUSSION

Of the 218 subjects, only 31 had to return a third time to obtain the 5% agreement and 4 a 4th time. If the data of the first 5 years by the second method had been based upon the average of only the first two mornings for all subjects, instead of including more than two mornings as was done for 17 of the subjects, the smoothed means would have been increased a small amount for each year; the differences, being less than ½ calorie in each case, are not statistically significant. The coefficient of correlation with age would remain unchanged; i.e., highly significant. Individual basal metabolisms would, however, have been significantly altered in some cases, 6 of the 17 cases which were recalculated for this purpose changing to levels which showed large differences.

When the smoothed means for each of the first 5 years, as obtained by the 2nd, 3rd, and 4th methods, are compared with those of the first, which is the lowest in each case, only the third shows a real difference for each of the 5 years, the results by the third method being higher in each case. The second showed no statistically significant difference for any of the years and the 4th none for the first 4 years, only the 18th year showing a better than even chance that the difference is real. Variability around the means is less by the second

method, as is demonstrated by the coefficients of variation for each year.

It would seem from the above that, for results obtained by the method of age-selected averages used here, the first two methods of evaluating individual tests would be most suitable in establishing basal metabolism standards. These data, at the time of writing, are being further analyzed on the basis of physical developmental levels and basal calories per day irrespective of age classification and independent of the method of averages (Wetzel, '44).

Comparison with other studies

Although methods used in determining basal metabolism are not the same in all studies, nevertheless some comparisons seem to be justified. As may be observed in figure 1, the basal metabolism of the younger subjects in the present study is fairly close to that of some of the studies reported from higher latitudes, but beginning at about the middle of the 15th year

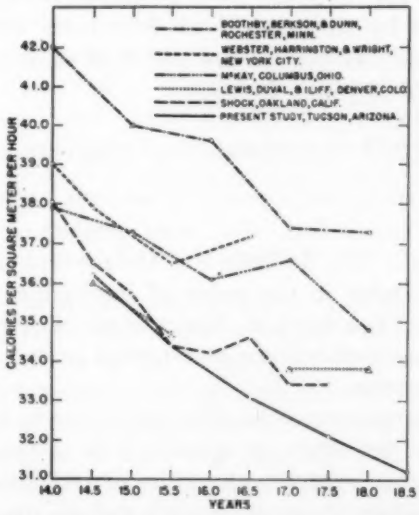

Fig. 1 True yearly means of the present study, as calculated by the first method of evaluating data, compared with other studies.

it rapidly approaches a much lower level. This is clearly demonstrated when comparison is made with the study of Shock ('42), whose subjects lived in Oakland, California. Naldandov, Heller, Krause and Purdy ('38), working in Oklahoma, reported that their younger girls had a rate of metabolism as high as those of the same age in the north, but that as they grew older Oklahoma girls showed a much sharper drop than northern girls.

Basal metabolism as reported from Florida by Tilt and Walters ('35) for girls 19 years of age and older is strikingly similar to that of southern Arizona for subjects of the same age, calories per square meter per hour of the former being 30.5, 31.4, 32.1, and 32.6 for the years 19, 20, 21, and 23, respectively. The study in the north which reported the largest number of cases is that of Boothby, Berkson and Dunn ('36). Using standards known as the Mayo Foundation standards which are based on first tests only, they predict 36.74 calories per m^2 per hour for the 19th year of age and 36.18 calories for the years 20 to 24, inclusive. In the present study, means by the method of first tests only are 31.00 calories for the 19th year of age and 32.06 calories for the age range of 20 to 23 years, inclusive.

Effect of environmental temperature

Five cooperating state universities have reported a study of the basal metabolism of a large number of college girls (Pittman et al., '43). In table 3 of the present study we have shown these states in the order of increasing mean annual temperatures (Yearbook of Agriculture, '41), together with the mean basal metabolisms as reported in each case for the 18-year-old groups.

Although temperature is only one factor to be considered in a study of the effect of climate, it is of interest to note that these states, with the exception of Oklahoma which has a limited number of cases, show a definite trend toward a diminishing basal level with increasing temperature.

The attainment of this low adult value beginning at the 18th year would seem to indicate the appearance of the chemical regulation mechanism in women in warm environments which was demonstrated by Hardy and DuBois ('40) and Hardy, Milhorat and DuBois ('41). These workers, studying the basal metabolism of 8 adult women in a room colorimeter with the direct method of calorimetry and a carefully regulated

TABLE 3

Basal metabolism of 18-year-old girls in midwestern states and in the present study

STATE	CASES	MEAN ANNUAL TEMPERATURE (1934–38 INC.)	CALORIES PER M^2 PER HOUR
Minnesota	34	41.6	35.7
Iowa	31	49.5	35.1
Ohio	40	52.0	33.8
Kansas	58	56.6	33.6
Oklahoma	19	61.7	34.0
Arizona, southern	36	67.2	31.2

temperature index (controlled barometric pressure, humidity, air movement, and environmental temperature), demonstrated the existence of a zone of minimal metabolism between 27°C. and 32°C. (81°F. and 90°F.). They reported a low mean of 30.9 calories per m^2 per hour in this 5-degree temperature range after an adaptation period of from two to three hours.

Effect of altitude

Although Tucson has an elevation of 2400 feet above sea level, it would seem improbable, from the results of the present study and a review of the literature, that altitude influences the basal metabolism provided one can discuss it properly separated from other factors contributing to the climatic effect. The studies of Lewis, Duval and Iliff ('43) support this observation. As shown in figure 1, Shock, working at the low altitude of Oakland, California, found higher mean values than those reported by us, and higher also than Lewis' 14-

and 15-year-old group studied at a much higher altitude in Denver, Colorado.

SUMMARY

1. Basal metabolism determinations have been made on 218 girls and young women of southern Arizona from 14 to 23 years of age, inclusive.

2. Calories per m^2 per hour were calculated by 4 methods of evaluating individual tests: (1) average of results of all tests within 5% of one accepted as a base line on at least two mornings, (2) average of all tests, (3) first tests only, (4) average of tests on first mornings only.

3. Smoothed yearly means in calories dropped sharply by all methods, by the first method from 36.40 to 31.21 for the years from 14 to 18, inclusive. Correlation with age was found to be highly significant statistically by the first two methods and less significant by the other two. When reducing the true yearly means to linear form, it was found that of the 4 methods of evaluating individual tests the best straight line fit was obtained by means of the second method and the next by the first. When the respective yearly means obtained by each of these two methods were compared, no statistically significant differences were found although those by the first were lower in each case. Variability around the means was less by the second method.

4. Lumped means of 31.1 and 31.5 calories were taken for the first two methods, respectively, for the years 18 to 23, inclusive. This low energy level would seem to indicate the appearance in women at this time of the zone of minimal metabolism demonstrated by Hardy, Milhorat and DuBois ('41).

5. When the 18-year-old group was compared with similar groups of the same age reported from 5 midwestern states, a diminishing metabolism was observed with increasing mean annual temperature.

6. The present study was made at an altitude of 2400 feet. If altitude per se tends to increase basal metabolism, as some

investigators have claimed, then the present low level of metabolism should not have been observed. Therefore our data do not support this view.

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MEAD JOHNSON AND COMPANY 'B-COMPLEX' AWARD

Nominations are solicited for the 1949 Award of $1000, established by Mead Johnson and Company to promote researches dealing with the B complex vitamins. The recipient of this Award will be chosen by a Committee of Judges of the American Institute of Nutrition and the formal presentation will be made at the annual meeting of the Institute in the spring of 1949.

The Award will be given to the laboratory (non-clinical) or clinical research worker in the United States or Canada who, in the opinion of the judges, has published during the previous calendar year, January 1 to December 31, the most meritorious scientific report dealing with the field of the 'B-complex' vitamins. While the award will be given primarily for publication of specific papers, the judges are given considerable latitude in the exercise of their function. If in their judgment circumstances and justice so dictate, it may be recommended that the award be made to a worker for valuable contributions over an extended period but not necessarily representative of a given year. Membership in the American Institute of Nutrition is not a requisite of eligibility for the award.

To be considered by the Committee of Judges, nominations for this award for work published in 1948 must be in the hands of the Chairman of the Nominating Committee by January 15, 1949. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate the task of the Committee of Judges in its consideration of the nomination.

HAROLD H. WILLIAMS
Cornell University, Ithaca, N. Y.

CHAIRMAN, NOMINATING COMMITTEE

BORDEN AWARD IN NUTRITION

Nominations are solicited for the 1949 Award of $1000, and a gold medal made available by the Borden Company Foundation, Inc. The American Institute of Nutrition will make this award in recognition of distinctive research by investigators in the United States and Canada which has emphasized the nutritive significance of the components of milk or of dairy products. The award will be made primarily for the publication of specific papers, but the judges may recommend that it be given for important contributions over an extended period of time. The award may be divided between two or more investigators. Employees of the Borden Company are not eligible for this honor.

The formal presentation will be made at the annual meeting of the Institute in the spring of 1949. To be considered for the award, nominations must be in the hands of the Chairman of the Nominating Committee by January 15, 1949. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate consideration for the award.

JAMES M. ORTEN
College of Medicine,
Wayne University,
Detroit, Michigan

CHAIRMAN, NOMINATING COMMITTEE

OSBORNE AND MENDEL AWARD

Nominations are invited for the Osborne and Mendel Award of $1000, established by the Nutrition Foundation, Inc., for the recognition of outstanding accomplishments in the general field of exploratory research in the science of nutrition. It shall be given to the investigator who, in the opinion of a Jury of Award, has made the most significant published contribution in the year preceding the annual meeting of the Institute, or who has published a series of contemporary papers of outstanding significance.

The Award will be presented at the annual meeting of the American Institute of Nutrition.

The recipient will be chosen by a Jury of Award of the American Institute of Nutrition. As a general policy, the Award will be made to one person. If, in the judgment of the Jury of Award, an injustice would otherwise be done, it may be divided among two or more persons. Normally preference will be given to research workers in the United States and Canada, but investigators in other countries, especially those sojourning in the United States or Canada for a period of time, are not excluded from consideration. Membership in the Institute of Nutrition is not a requirement for eligibility and there is no limitation as to age.

Nominations may be made by anyone. Nominations for the 1949 Award, accompanied by data relative to the accomplishments of the nominee, must be sent to the Chairman of the Nominating Committee before January 15, 1949.

D. W. Woolley
Rockefeller Institute for Medical Research, New York, N. Y.

Chairman, Nominating Committee